CHRONIC GIARDIASIS IN CBA/N MICE: USE OF GENETICALLY IMMUNODEFICIENT MICE TO STUDY MECHANISMS OF IMMUNITY TO AN INTESTINAL PARASITE

BY

DANNA LYNN SKEA, B.Sc., M.Sc.

A Thesis

Submitted to the School of Graduate Studies

in Partial Fulfilment of the Requirements

for the Degree of

Doctor of Philosophy

McMaster University

August, 1989

CHRONIC GIARDIASIS IN CBA/N MICE

DOCTOR OF PHILOSOPHY (1989)
(Medical Sciences)

MCMASTER UNIVERSITY
Hamilton, Ontario

TITLE: Chronic Giardiasis in CBA/N Mice: Use of Genetically Immunodeficient Mice to Study Mechanisms of Immunity to an Intestinal Parasite.

AUTHOR: Danna Lynn Skea, B.Sc. (University of Toronto)
M.Sc. (University of Toronto)

SUPERVISOR: Professor Brian J. Underdown

NUMBER OF PAGES: xiii, 168

Abstract

Giardia muris is an intestinal parasite of mice. It has a simple life cycle and is non-invasive. Therefore, G. muris infection provides a model to study immune mechanisms that operate at mucosal surfaces. Immunocompetent mice eliminate primary G. muris infections. T cell-dependent humoral immune mechanisms are involved in this process.

The CBA/N mouse bears an X-linked immunodeficiency gene (Xid), the expression of which results in defective B cell maturation and consequent impairment of certain humoral immune responses. The antibody responses of CBA/N mice are particularly defective in certain isotypes and specificities.

CBA/N mice fail to eliminate G. muris. The major focus of this dissertation was an attempt to elucidate the basis for chronic giardiasis in this strain.

Cellular reconstitution experiments showed that the ability to eliminate G. muris was transferred to CBA/N mice with lymphoid cells from immunocompetent, CBA/Ca mice. Reconstitution required prior irradiation of recipient mice, and was not effective with semi-purified B cells and T cells. These results indicate that conventional B cells and T cells are insufficient, and that another cell type is also required. This cell may be the Lyl+ B cell.

CBA/N mice make quantitatively deficient serum IgG antibody responses to G. muris infection. Providing CBA/N mice with this antibody failed to induce elimination of the parasite, thus this

isotype defect was ruled out as the cause of their susceptibility to chronic giardiasis.

Analysis of G. muris antigen recognition failed to reveal a specificity defect in the antibody response of CBA/N mice. However, a glycolipid component of G. muris bound serum IgM from CBA/J and BALB/c mice, but not serum IgM from CBA/N mice. These results indicate a possible structural defect in IgM from CBA/N mice.

Although unable to eliminate primary G. muris infection, drug-cured CBA/N mice are resistant to reinfection. These results indicate that the immune mechanisms that mediate elimination of G. muris are different from those that mediate resistance to reinfection.

Acknowledgements

I thank Dr. Brian J. Underdown for his supervision of this work. His patience, guidance and enthusiasm were much appreciated.

I thank the members of my supervisory committee, Dr. William Rawls, Dr. Kenneth Rosenthal and Dr. John Bienenstock, and also Dr. Peter Ernst, for their valuable advice.

I thank Iain Switzer, Dawn Stetsko and Alan Stafford for their technical assistance, and Barb Krick for the kind care of the animals used in this research.

I thank Susan Evans, Denis Shaw, Rona Madison, Dawn Stetsko, Alan Stafford, Dina Ozols, Iain Switzer, Donny Gasangwa, Sharon Molnar and Ken Rosenthal for their friendship.

I thank my family, especially my parents, Lieutenant-Colonel and Mrs. Stanley J. Skea, for their emotional and financial support, and I thank my nieces, Sarah, Amanda and Rebecca, and my nephew, Matthew.

This work was supported by a studentship from the Medical Research Council of Canada.

1.4.	**Giardiasis in CBA/N Mice**	29
1.4.1.	Hypotheses Concerning the Basis for Chronic Giardiasis in CBA/N Mice	29
1.4.2.	Resistance to Reinfection with *G. muris*	31
CHAPTER 2	**METHODS AND MATERIALS**	
2.1.	Mice	32
2.2.	Infection of Mice with *G. muris*	32
2.3.	Isolation and Counting of *G. muris* Cysts	33
2.4.	Drug Treatment of *G. muris*-infected Mice	33
2.5.	Isolation of *G. muris* Trophozoites	34
2.6.	Collection of Sera	34
2.7.	Adoptive Transfer of Cells Between Mice	35
2.7.1.	Cell Suspensions	35
2.7.2.	T cell Depletion	35
2.7.3.	B cell Depletion	36
2.7.4.	Monitoring of T cell and B cell Depletions	37
2.7.4.1.	F.A.C.S.	37
2.7.4.2.	Mitogenic Stimulation of Cells	37
2.7.5.	Reconstitution of Mice	39
2.8.	Immunization of Mice with *G. muris* Trophozoites	40
2.9.	Passive Transfer of anti-*G. muris* Antisera to CBA/N Mice	40
2.10.	Enzyme-Linked Immunosorbent Assay for Total Serum IgM	41
2.10.1.	Coating Antibody	41
2.10.2.	Assay Procedure	42
2.10.3.	Specificity	43

2.10.4. Standard Curve and Determination of Serum IgM Levels 44

2.11. Analysis of the Antibody Response to a Type II T-independent Antigen 44

2.11.1. ELISA 44

2.12. Immunoradiometric Assay for IgG anti-G. muris Antibody 48

2.12.1. Coating Antigen 48

2.12.2. Assay Procedure 48

2.12.3. Specificity 49

2.12.4. Standard Curve and Determination of Antibody Activity 49

2.13. Analysis of the Lipid Components of G. muris 50

2.13.1. Extraction of Lipids from G. muris 50

2.13.2 Dot Blot Assay 51

2.13.3. Thin Layer Chromatography and Immunoblotting 52

2.14. SDS-PAGE and Western Blotting 53

2.14.1. Preparation of Antigens 53

2.14.2. SDS-PAGE 54

2.14.3. Western Blotting 55

2.15. Statistics 56

CHAPTER 3 RESULTS AND DISCUSSION

3.1 Adoptive Transfer of Cells to CBA/N Mice to Study the Cellular Requirements for Elimination of G. muris 57

3.1.1. Results 60

3.1.1.1. Reconstitution of Non-irradiated CBA/N Mice with Spleen Cells from CBA/Ca Mice 60

3.1.1.2. Reconstitution of Non-irradiated CBA/N Mice with Peyer's Patch and Mesenteric Lymph Node Cells from CBA/Ca Mice 61

3.1.1.3. Reconstitution of Irradiated CBA/N and CBA/Ca Mice with Spleen Cells from CBA/N and CBA/Ca Mice .. 65

3.1.1.4. Reconstitution of Irradiated CBA/N Mice with Mixtures of Splenic B cells and T cells from CBA/N and CBA/Ca Mice 67

3.1.2. Discussion 76

3.1.3. Suggestions for Future Experiments 85

3.2. Passive Transfer of Serum and Immunization of CBA/N Mice to Study the Effect of IgG Antibody on Elimination of G. muris 87

3.2.1. Results 90

3.2.1.1. Comparison of the IgG Antibody Response to G. muris Infection in CBA/N and CBA/Ca Mice 90

3.2.1.2. Active Immunization of CBA/N and CBA/J Mice with G. muris Trophozoites 90

3.2.1.3. Passive Transfer of Hyperimmune Anti-G. muris Antisera from CBA/J Mice to CBA/N Mice 93

3.2.2. Discussion 100

3.3. Analysis of G. muris Antigen Recognition by Antibodies from CBA/N Mice 105

3.3.1. Results 107

3.3.1.1. Western Blot Analysis of G. muris Protein and Glycoprotein Antigens 107

3.3.1.2. Analysis of G. muris Lipid and Glycolipid Antigens 116

3.3.2. Discussion 126

3.4. Resistance of CBA/N Mice to Reinfection with G. muris 132

3.4.1. Results 134

3.4.1.1. Resistance of CBA/N and CBA/Ca Mice to Reinfection with G. muris After Drug-cure of a Primary Infection 134

3.4.1.2. Reconstitution of Irradiated CBA/N and CBA/Ca Mice with Spleen Cells from G. muris-immune CBA/N CBA/Ca Mice 137

3.4.2. Discussion 143

CHAPTER 4 CONCLUSIONS 148

References 155

List of Figures

Figure		Page
1.	Standard Curves for Solid Phase Assays	46
2.	Adoptive Transfer of (A) Spleen Cells and (B) Peyer's Patch and Mesenteric Lymph Node Cells to Non-irradiated CBA/N Mice: Course of G. muris Infection in Reconstituted Mice	63
3.	Adoptive Transfer of Spleen Cells to Irradiated CBA/N and CBA/Ca Mice: Course of G. muris Infection in Reconstituted Mice	69
4.	Adoptive Transfer of Mixtures of T cell-depleted and B cell-depleted Splenocytes from CBA/N and CBA/Ca Mice to Irradiated CBA/N Mice: Course of G. muris Infection in Reconstituted Mice	73
5.	Immunization of Mice with G. muris Trophozoites: Course of G. muris Infection in Immunized and Sham-Immunized Mice	97
6.	Passive Transfer of Hyperimmune Anti-G. muris Antisera to CBA/N Mice: Course of G. muris Infection in Recipient Mice	99
7.	Western Blot Analysis of G. muris Trophozoite Antigens Recognized by Hyperimmune Anti-G. muris Antisera from CBA/N and CBA/J Mice	109
8.	Western Blot Analysis of G. muris Trophozoite and Cyst Antigens Recognized by Serum IgG Antibodies from G. muris-infected CBA/N and CBA/Ca Mice	112
9.	Western Blot Analysis of G. muris Trophozoite and Cyst Antigens Recognized by Serum IgA Antibodies from G. muris-infected CBA/N and CBA/Ca Mice	113
10.	Western Blot Analysis of G. muris Trophozoite and Cyst Antigens Recognized by Serum IgM Antibodies from G. muris-infected CBA/N and CBA/Ca Mice	114
11.	Dot Blot Analysis of Chloroform:Methanol Extract of G. muris Trophozoites: Investigation of Binding by Immunoglobulin Isotypes from CBA/N and CBA/J Mice	119
12.	Dot Blot Analysis of Chloroform:Methanol Extract of G. muris Trophozoites: Investigation of Binding by IgM from CBA/N, CBA/J and BALB/c Mice	123

13. Thin Layer Chromatography of Chloroform:Methanol Extract of G. muris Trophozoites 125

14. Susceptibility of CBA/N Mice to a Secondary Challenge Infection with G. muris Following a Primary Infection of Three Weeks' Duration 139

15. Adoptive Transfer of Spleen Cells From G. muris-immune CBA/N and CBA/Ca Mice: Course of G. muris Infection Reconstituted Mice 141

List of Tables

Table		Page
1.	T cell and B cell Depletions from Spleen Cell Suspensions ...	38
2.	Reconstitution of CBA/N Mice with Spleen Cells and Peyer's Patch and Mesenteric Lymph Node Cells from CBA/Ca Mice ...	64
3.	Reconstitution of Irradiated Mice with CBA/N and CBA/Ca Spleen Cells	70
4.	Reconstitution of Irradiated CBA/N Mice with Mixtures of T cell-depleted and B cell-depleted Spleen Cells from CBA/N and CBA/Ca Mice	74
5.	Serum IgG Antibody Response of CBA/N and CBA/Ca Mice to Infection with *G. muris*	91
6.	Serum IgG Anti-*G. muris* Antibody Levels in Actively and Passively Immunized CBA/N Mice	92
7.	Resistance of CBA/N Mice to a Secondary Challenge Infection with *G. muris*: Dependence on Duration of Primary Infection	135

Chapter 1

INTRODUCTION

1.1 Objectives and Experimental Approaches

The mucosal tissues of the body, comprising the respiratory, genitourinary and gastrointestinal tracts, are portals of entry for, and major sites of infection of potentially pathogenic microorganisms. Therefore, the mucosal immune system is an important line of defence for protection against disease, and immunization at mucosal surfaces provides a powerful strategy for the prevention of infection (Befus and Bienenstock 1980). The production of vaccines designed to stimulate protection against mucosal infections requires an understanding of the molecular and cellular mechanisms involved in mucosal immunity. The objective of the work described in this thesis was to utilize a genetic model of immunodeficiency to study the mechanisms of immunity to an intestinal parasite of mice.

The parasite that was used was Giardia muris. The non-invasive nature of this parasite (Owen, Nemanic and Stevens 1979) and its simple life cycle (Cheng 1986) make murine giardiasis a valuable model for the study of immune mechanisms that operate at the external surface of mucosal tissues (Underdown et al. 1988).

The CBA/N mouse bears an X-linked immunodeficiency gene (Xid), the expression of which results in defective B cell maturation and consequent impairment of certain humoral immune responses (Scher 1982a). Unlike most immunocompetent mouse strains which eliminate G. muris infection within 6 to 10 weeks (Roberts-Thomson and Mitchell 1978), mice of the CBA/N strain fail to eliminate this parasite (Snider, Skea and Underdown 1988). Therefore, CBA/N mice are considered to be susceptible to chronic giardiasis. Thus, the CBA/N mouse model of B cell deficiency was used to investigate the immune mechanisms that are important for elimination of G. muris infection.

The question that is central to this dissertation is "Why do CBA/N mice fail to eliminate G. muris ?". My primary hypothesis was that the B cell defect of CBA/N mice renders them susceptible to chronic giardiasis. A number of strategies were employed to test this hypothesis. Investigation of the defect expressed at the cellular level was carried out by adoptive transfer of lymphoid cells from immunocompetent mice to CBA/N mice, and the subsequent assessment of the course of G. muris infection in the recipient mice. At the molecular level, the isotypes and specificities of antibodies thought to be required for elimination of G. muris were investigated.

It was previously shown that CBA/N mice make a quantitatively defective serum IgG antibody response to infection with G. muris (Snider, Skea and Underdown 1988). Although it did not seem likely that serum IgG antibody would be important for elimination of a mucosal infection, I, nevertheless, tested this hypothesis using the following strategies. Large quantities of serum IgG anti-G. muris antibodies were provided to

CBA/N mice by passive transfer of hyperimmune anti-G. muris antisera, and by active, parenteral immunization using the isolated parasite and an adjuvant. The influence of the presence of serum IgG antibody on the course of G. muris infection was thus assessed.

Another hypothesis was that CBA/N mice have a specificity defect in their antibody response to G. muris. That is, they may fail to recognize a crucial antigen or antigens on the parasite that stimulate immune elimination in immunocompetent strains of mice. The methods used to test this hypothesis included Western blotting, to examine protein and glycoprotein antigen recognition. In addition, apolar components of the parasite were extracted and examined by dot blot analysis and thin layer chromatography, to investigate lipid and glycolipid antigen recognition. The antigen recognition pattern of antibodies from CBA/N mice was compared to that of antibodies from immunocompetent mice which eliminate G. muris.

It is known that the immune mechanisms that mediate elimination of a primary infection are not necessarily the same as those that mediate resistance to reinfection with the same infectious agent (Mitchell, McMichael and Lamb 1985). The question of whether, in the case of G. muris infection, these mechanisms are the same or different was addressed by assessing the resistance of CBA/N mice to reinfection with G. muris, after drug-cure of a primary infection.

The use of a genetic model of immunodeficiency to identify protective immune mechanisms is limited by the possibility that protective immunity may require the cooperation of several immune elements. Thus, the identification of one immune element may reflect its importance, but will not rule out the importance of other immune elements. The work

described in this thesis was an attempt to assess the requirement for B cells and antibody in the elimination of the intestinal parasite, G. muris. It may be that antibody cooperates with other immune elements to provide protective immunity against G. muris infection. To obtain a complete understanding of how the mucosal immune system mediates protection against infectious disease, the contribution and cooperation of all of the relevant immune elements must be considered.

1.2 Giardiasis in Mice

1.2.1 Giardia muris: Morphology, Life Cycle and Infection in Mice

Giardia muris is a flagellated, protozoan parasite which infects the upper small intestine of mice (Cheng 1986). The organism exists in two forms: the trophozoite is the vegetative form which replicates by binary fission, and the cyst is the infectious form which is resistant to environmental stresses. The pear-shaped trophozoite is 9-12 μm long and 5-7 μm wide; its dorsal surface is convex, and its ventral surface, concave. This concavity forms an adhesive (sucking) disc on the anterior half of the ventral surface of the trophozoite. In addition, the trophozoite possesses four pairs of flagella and two nuclei. The elliptically-shaped cyst is 6-10 μm long and is surrounded by a cyst wall which is 0.3-0.5 μm thick. The cyst contains trophozoite structures and two to four nuclei. The morphology of G. muris trophozoites and cysts has been reviewed, in detail, by Feely, Erlandsen and Chase (1984).

Giardia muris infection begins when a host organism ingests G. muris cysts (Roberts-Thomson et al. 1976a). Upon passage through the acidic contents of the stomach, excystation occurs; this process involves the chemical breakdown of the cyst wall and the emergence of trophozoites (Bingham and Meyer 1979). The trophozoites feed and reproduce in the

proximal half of the small intestine, where they remain close to the luminal wall and attached to the epithelial surface (Owen, Nemanic and Stevens 1979). The attachment process is thought to involve mechanical forces generated by the ventral sucking disc (Erlandsen and Feely 1984), as well as specific membrane receptors which bind to molecules present on epithelial cells (Inge, Edson and Farthing 1988). Mucosal penetration by trophozoites is rare and usually requires pre-existing damage to the mucosal epithelium (Owen, Nemanic and Stevens 1979). Thus, G. muris is considered to be a non-invasive parasite. The exact nature of the signals for detachment of trophozoites from the epithelial surface, and their subsequent encystation in the proximal small intestine, is unknown. However, small intestinal factors, including slightly alkaline pH, bile salts and fatty acids, are thought to be involved (Gillin et al. 1987; Gillin, Reiner and Boucher 1988). The newly formed cysts, which complete the life cycle of the parasite, are excreted in the feces, and persist in the environment, ready to infect another host.

Experimental administration of G. muris cysts to mice results in a reproducible pattern of infection (Belosevic and Faubert 1983a). Following the inoculation, there is a short latent period of 3-10 days before the infected mice begin to excrete cysts in their feces (Belosevic et al. 1984). Maximal cyst output occurs at 1-2 weeks of infection, corresponding to the period of maximal parasite (trophozoite) burden in the intestine (Roberts-Thomson et al. 1976a; Olveda, Andrews and Hewlett 1982). Cyst output and trophozoite burden gradually decrease thereafter, and most immunocompetent strains of mice eliminate the parasite within 5-10 weeks of infection (Belosevic et al. 1984). Following a primary

infection with G. muris, immunocompetent mice are resistant to re-infection with the parasite (Roberts-Thomson et al. 1976b).

Giardiasis in the mouse is a relatively mild infection. In immunocompetent mice, the pathological consequences of G. muris infection include impaired weight gain (Roberts-Thomson et al. 1976a), as well as histological and biochemical changes in the small intestine. Histologically, giardiasis was found to cause a transient reduction in the villous:crypt ratio in jejunal segments of the small intestine (Roberts-Thomson et al. 1976a; Olveda, Andrews and Hewlett 1982; Brett and Cox 1982). Biochemically, the infection was found to result in a transient reduction in jejunal brush border disaccharidase activities (Gillon, Thamery and Ferguson 1982). In immunodeficient, athymic (nude) mice, giardiasis is thought to contribute to the post-weaning, wasting syndrome that leads to early mortality in this strain (Boorman et al. 1973).

Although G. muris infection is largely restricted to rodent hosts (Meyer 1985), a closely related parasite, Giardia lamblia, infects humans (Nash et al. 1987). G. lamblia is transmitted by contaminated water supplies (Moore et al. 1969), food (Osterholm et al. 1981) and person-to-person contact (Black et al. 1977). The prevalence of human giardiasis has been estimated to be between 1 and 7% in industrialized countries, and as high as 50% in some developing countries (Dupont and Sullivan, 1986). Clinically, G. lamblia infection is associated with a wide spectrum of illness, ranging from asymptomatic infection to acute diarrhea to chronic and recurrent gastrointestinal illness (Pickering 1985). Thus, human giardiasis is considered to be an important public health issue. G. muris infection in mice, therefore, not only provides a model for the study of

mucosal immunity, but also provides an animal model of a human disease (Roberts-Thomson et al. 1976a).

1.2.2 Immunity to G. muris

1.2.2.1 In Vivo Studies of the Cellular Requirements for Elimination of G. muris

Most immunocompetent strains of mice eliminate a primary infection with G. muris within 5 to 10 weeks (Roberts-Thomson and Mitchell 1978; Belosevic et al. 1984). However, athymic (nude) mice failed to eliminate the parasite, developing instead a chronic infection characterized by continuous output of large numbers of fecal cysts (Roberts-Thomson and Mitchell 1978; Stevens, Frank and Mahmoud 1978). Nude mice that were reconstituted with lymphoid cells from thymus-intact mice eliminated G. muris (Roberts-Thomson and Mitchell 1978). These results indicate that thymus-dependent mechanisms of immunity, that is, T lymphocytes, are crucial for the elimination of the parasite.

Mice treated with monoclonal anti-L3T4 antibody are deficient in the class II restricted, helper subset of T lymphocytes; these mice failed to eliminate G. muris (Heyworth, Carlson and Ermak 1987). Mice treated with monoclonal anti-Lyt2 antibody are deficient in the class I restricted, cytotoxic and suppressor subsets of T lymphocytes; these mice eliminated G. muris infection in a normal manner (Heyworth, Carlson and Ermak 1987). Beige mice, which are genetically deficient in natural killer cell activity, also eliminated G. muris (Heyworth, Kung and

Eriksson 1986). The results of these studies indicate that cytotoxic cells do not play a crucial role in the elimination of G. muris by immunocompetent mice. However, helper T cells appear to be crucial for immune elimination of the parasite.

Mice treated from birth with anti-IgM antisera are deficient in B cells and immunoglobulin; these mice failed to eliminate G. muris (Snider et al. 1985). It was shown that, in contrast to untreated mice, which eliminated the parasite, the anti-IgM antisera-treated mice did not make specific anti-G. muris antibody in response to G. muris infection.

Taken together, the results of these studies indicate that T cell-dependent, humoral immune mechanisms are crucial for elimination of G. muris .

W^v/W^v mice, which are genetically deficient in mast cells, failed to eliminate G. muris (Erlich et al. 1983). However, reconstitution of these mice with bone marrow cells from intact mice, failed to reconstitute the ability to eliminate the parasite, even though mast cells were detected in the reconstituted mice. These results have not been confirmed, and the reason for chronic infection in this strain remains uncertain.

In vivo studies of susceptibility to a pathogen, following the selective depletion of a particular cell type, are useful in identifying immune elements that are important for resistance to that pathogen. However, such a negative selection approach may fail to identify other elements of the immune system that cooperate with the identified elements. For example, specific antibody that binds to G. muris may subsequently interact with effector elements that mediate elimination of the antibody-

parasite complex. Therefore, it is important to also study immune responses to a pathogen in immunologically intact animals.

1.2.2.2 Humoral Immunity in Giardiasis

Studies of the humoral immune response to G. muris infection in immunocompetent mice have quantitatively and temporally defined the production of specific anti-parasite antibodies (Andrews and Hewlett 1981; Anders, Roberts-Thomson and Mitchell 1982; Underdown et al. 1982; Snider et al. 1985; Snider and Underdown 1986; Heyworth 1986). IgA and IgG antibodies were detected in the sera of infected mice. More importantly, secretory IgA antibodies were detected in intestinal secretions of infected animals (Anders, Roberts-Thomson and Mitchell 1982; Snider and Underdown 1986). There was a temporal association between the intestinal IgA antibody response to G. muris infection and the elimination of the parasite (Snider and Underdown 1986). There was a similar association between the serum IgG antibody response and elimination of G. muris. The serum IgA antibody response occurred slightly later than the intestinal response.

A sensitive, immunoradiometric assay failed to detect antibody of the IgG isotype in intestinal secretions of infected, immunocompetent mice (Snider and Underdown 1986). However, in a different study, immunofluorescence of trophozoites obtained from the intestines of similar mice revealed the presence of both IgA and IgG antibodies bound to the parasite (Heyworth 1986). Trophozoites obtained from the intestines of nude mice did not have attached antibody.

The suckling offspring of immune mice are resistant to infection with G. muris (Stevens and Frank 19[illegible]). The milk produced by immune mothers was shown, by indirect immunofluorescence, to contain IgA and IgG anti-G. muris antibodies (Andrews and Hewlett 1981).

Since G. muris is a lumen-dwelling parasite (Owen, Nemanic and Stevens 1979), intestinal antibody is more likely than serum antibody to be involved in its elimination (Snider and Underdown 1986). Several mechanisms by which antibody may mediate elimination of G. muris have been proposed. Antibody bound to the surface of the trophozoite may restrict its mobility, or prevent its attachment to the epithelial surface, or both (Loftness et al. 1984; Belosevic and Faubert 1987; Inge, Edson and Farthing 1988). In addition, antibodies may agglutinate trophozoites (Butscher and Faubert 1988). Such immune interference with normal activities of the parasite may contribute to its elimination from the intestine. Direct killing of trophozoites by antibody, as was shown, in vitro, by Nash and Aggarwal (1986), may also contribute to the elimination of G. muris infection.

Alternatively, specific anti-G. muris antibody may cooperate with effector elements of the immune system to achieve elimination of the parasite. Several authors have described killing of Giardia trophozoites, in vitro, by antibody and complement (Hill, Burge and Pearson 1984; Belosevic and Faubert 1987; Deguchi, Gillin and Gigli 1987). However, these studies employed serum, rather than secretory antibodies, and the presence of the complement system in the intestinal lumen is questionable (Colten 1974). Nevertheless, it was suggested (Hill, Burge and Pearson

1984) that antibody and complement may play a role in preventing invasion by trophozoites beyond the intestinal epithelium.

Antibody may also cooperate with effector cells in an immune attack against G. muris infection. Many in vitro studies have shown that specific anti-Giardia antibodies enhanced killing of trophozoites by phagocytic cells (Radulescu and Meyer 1981; Smith, Keister and Elson 1983; Smith et al. 1984; Kaplan et al. 1985; Belosevic and Faubert 1986a; Hill and Pearson 1987). Opsonic adherence of trophozoites was demonstrated with both macrophages and neutrophils. Destruction of the ingested parasites was confirmed by microscopic examination of trophozoite morphology. Although most of these studies employed serum antibodies, one study (Kaplan et al. 1985) showed that secretory IgA antibodies, derived from milk of immune mice, opsonized trophozoites for ingestion by phagocytes. The cells employed in these studies were derived from peripheral blood or the peritoneal cavity. However, phagocytic cells are known to be present in the intestine (Owen, Allen and Stevens 1981; LeFevre, Hammer and Joel 1982; Heyworth et al. 1985a). In addition, Kanwar et al. (1986) demonstrated that serum antibody enhanced killing of trophozoites by lymphocytes derived from the lamina propria of mice. Thus antibody-dependent, cell-mediated cytotoxicity may be an important immune mechanism in the elimination of G. muris infection, in vivo.

1.2.2.3 Cellular Immunity in Giardiasis

Macrophages can kill G. muris trophozoites, in vitro, even in the absence of specific antibody (Radulescu and Meyer 1981; Kaplan et al, 1985; Hill and Pearson 1987). Smith et al. (1984) showed that peritoneal macrophages from C3H/HeN mice exhibited spontaneous, as well as antibody dependent, cytotoxicity for Giardia trophozoites. Belosevic and Faubert (1986b) showed that peritoneal macrophages from A/J mice (which required more than 10 weeks to eliminate G. muris) were less responsive to non-specific stimulation than macrophages from B10.A mice (which eliminated G. muris in less than 6 weeks). However, macrophages from the two strains of mice were equally effective at killing G. muris trophozoites, in vitro (Belosevic and Faubert 1986a).

Heyworth et al. (1985a; 1985b) demonstrated the presence of macrophages in the intestinal lumen of G. muris-infected mice. The number of luminal macrophages was the same in infected and non-infected mice. However, in the intestinal lavage fluids from infected mice, some macrophages were observed to be attached to trophozoites.

Owen, Allen and Stevens (1981) used electron microscopy to examine the cellular response of Peyer's patches to G. muris infection. They showed that macrophages, located beneath the basal lamina, extended pseudopods through the epithelium, and trapped, engulfed and then degraded trophozoites. In immunocompetent mice, macrophages that had phagocytosed G. muris were closely associated with lymphoblasts. These observations suggest that Peyer's patch macrophages provide two anti-Giardia functions,

in vivo: (1) they kill trophozoites, and (2) they present G. muris antigens to lymphocytes to initiate a specific anti-parasite immune response. In nude mice, enhanced and prolonged phagocytosis of trophozoites was observed. Nude mouse macrophages that had phagocytosed G. muris were not associated with lymphoblasts. This result is consistent with the lack of T lymphocytes in these mice. Since nude mice fail to eliminate G. muris infection (Roberts-Thomson and Mitchell 1978), these results further indicate that, while macrophages are probably important in controlling G. muris infection, they are, by themselves, insufficient to mediate elimination of the parasite.

A possible role for cytotoxic T lymphocytes in immunity to G. muris has been considered. However, the available evidence does not support such a role. Lamina propria lymphocytes were shown to kill trophozoites, in vitro, (Kanwar et al. 1986). However, the lymphocyte subset was not identified, and the killing was enhanced by antibody. In an early electron microscopy study Owen, Nemanic and Stevens (1979) reported the observation of intraluminal lymphocytes in close association with trophozoites. However, in a subsequent study (Owen, Allen and Stevens 1981), this group reported that there was no direct contact between lymphocytes and trophozoites in Peyer's patches. Similarly, in a preliminary study, Heyworth et al. (1985a) reported the observation of lymphocytes attached to trophozoites in intestinal lavage fluid from G. muris-infected mice. In a subsequent study (Heyworth, Owen and Jones 1985b), this group reported that lymphocytes were not attached to trophozoites. These results do not support a role for cytotoxic T-lymphocyte-mediated immunity to G. muris infection since the observations

were not reproducible, the lymphocyte populations were not defined, and the possible participation of antibody was not considered.

Several investigators have attempted to correlate numbers of cells with immunity to G. muris. Approximately 40% of the leukocytes recovered by intestinal lavage of immunocompetent mice were identified as T cells (Heyworth, Owen and Jones 1985b). In nude mice, this value was much lower. As expected, nude mice were particularly deficient in L3T4+ (helper) T cells. However, the numbers of luminal Lyt2+ (cytotoxic/suppressor) T cells were the same in immunocompetent and nude mice. In this study, a small increase in the number of luminal leukocytes was observed in G. muris-infected mice, compared to non-infected mice. However, in another study (Heyworth et al. 1985a), similar numbers of luminal leukocytes were found in infected and non-infected mice.

Carlson, Heyworth and Owen (1986a; 1986b) showed that the total number of leukocytes in Peyer's patches of immunocompetent mice doubled during G. muris infection, and then returned to normal levels after elimination of the parasite. The relative proportions of T cell subsets and B cells were not altered during the infection. However, these investigators noted a shift from IgM-bearing B cells to IgA-bearing B cells late in the infection.

Several groups have reported that the number of intraepithelial leukocytes in mice increased approximately 2-fold during infection with G. muris, and remained elevated after the parasite had been eliminated (MacDonald and Ferguson 1978; Brett and Cox 1982; Gillon, Thamery and Ferguson 1982). Intraepithelial leukocytes constitute a heterogenous group of cells, including a large proportion of granulated, lymphocyte-

like cells (Ernst et al. 1985). The immunological significance of intraepithelial leukocytes in giardiasis is unknown.

In summary, intestinal macrophages are likely to be involved in the elimination of G. muris. The available evidence indicates that macrophages present G. muris antigens to lymphocytes, and participate in the destruction of trophozoites. However, macrophages, alone, are insufficient for the elimination of G. muris. There is little evidence to support the hypothesis that cytotoxic T lymphocytes mediate elimination of G. muris. The observation that mice, depleted of Lyt2+ T cells, eliminated the parasite in a normal manner, is inconsistent with this hypothesis. The role of intraepithelial leukocytes remains uncertain.

1.2.2.4 G. muris Antigens

Very little is known about the antigens of G. muris to which protective immune responses are directed.

Erlich et al. (1983) biosynthetically labelled G. muris trophozoites and conducted immunoprecipitation using sera from immunized and infected BALB/c mice (which eliminated the parasite) and C3H/He mice (which developed chronic infection). Some antigens were better recognized by antibodies from the BALB/c mice than by antibodies from the C3H/He mice. These included an 82K protein and a complex of four, acidic, 32K proteins which bound to wheat germ agglutinin and were collectively called "Gm32". Although the ability to recognize these antigens was associated with the ability to eliminate the parasite in parental mice, in (BALB/c X C3H/He) F2 mice, this association was not consistent: some F2 mice

produced antibodies against these antigens, but developed chronic giardiasis.

More work has been done with the human parasite, G. lamblia. A large number of G. lamblia antigens have been described by investigators who used immunoprecipitation and immunoblotting techniques that employed patient sera and rabbit anti-G.lamblia antisera (reviewed in den Hollander, Riley and Befus 1988). A number of investigators have described a major, surface, glycoprotein of G. lamblia that may correspond to the 82K, G. muris antigen described by Erlich et al. (1983) (Einfield and Stibbs 1984; Edson et al. 1986; Clark and Holberton 1986; Kumkum et al. 1988). In addition, wheat germ agglutinin-binding to G. lamblia has been reported (Hill, Hewlett and Pearson 1981). However, the G. lamblia components that bound this lectin were larger than the G. muris, Gm 32 antigens described by Erlich et al. (1983) (Ward et al. 1988).

Of particular interest is the G. lamblia lectin, taglin, which has been described (Farthing, Pereira and Keusch 1986; Lev et al. 1986; Ward et al. 1987). This molecule, which is most specific for the saccharide, mannose-6-phosphate, bound to mouse enterocytes after it was activated by the intestinal protease, trypsin (Lev et al. 1986). Thus, taglin may be involved in the attachment of trophozoites to the intestinal epithelium. As such, this antigen may provide an important target for a host-protective immune response. Whether G. muris trophozoites possess a similar component is unknown.

1.2.2.5 Summary

In vivo studies of the cellular requirements for elimination of *G. muris* infection indicate that T cell-dependent humoral immune mechanisms are crucial. Since *G. muris* is an extracellular, lumen-dwelling parasite, secretory IgA antibody is thought to be important. This antibody may work alone or in cooperation with other immune elements to achieve elimination of the parasite. To further elucidate the mechanisms of immunity that contribute to elimination of *G. muris* infection, I have used the CBA/N mouse model of B cell deficiency, since it is known that CBA/N mice fail to eliminate *G. muris*.

1.3 The CBA/N Mouse: A Genetic Model of B cell Deficiency

The CBA/N mouse bears an X-linked, recessive gene (Xid), the expression of which results in defective B cell maturation with the consequent impairment of certain humoral immune responses (Amsbaugh et al. 1972; Scher et al. 1975a; 1975b; Scher 1982b; Huber 1982). The initial identification of immune defectiveness in this strain was based on the inability of these mice to respond to type II T-independent antigens, such as trinitrophenyl Ficoll (TNP-Ficoll) (Scher et al. 1975a). Subsequent studies of the Xid defect compared CBA/N mice to immunocompetent mice, such as CBA/Ca and BALB/c mice. Other studies compared immune defective F1 male offspring of crosses between CBA/N mice and immunocompetent mice to phenotypically normal F1 female offspring of such crosses. For simplicity, in the following literature review, CBA/N mice and immune defective F1 male offspring are referred to as "Xid mice", while their immunocompetent counterparts are referred to as "normal mice".

B lymphocytes from normal, adult mice are classified into two broad categories on the basis of their phenotype (Huber 1982): (1) mature B cells which bear the differentiation markers Lyb3 (Huber, Gershon and Cantor 1977), Lyb5 (Ahmed et al. 1977), and Lyb7 (Subbarao et al. 1979), and which have a low surface IgM/IgD ratio (Hardy et al. 1982), and (2) immature B cells which lack these differentiation markers and have a high

surface IgM/IgD ratio. The mature B cell subset (Lyb3+, Lyb5+, Lyb7+, low surface IgM/IgD), which is absent in normal, neonatal mice, is also absent in adult, Xid mice. The absence of mature B cells indicates that there is a block in the differentiation pathway of B cells in Xid mice. Consequently, Xid mice possess significantly fewer B lymphocytes than normal, adult mice (Sprent et al. 1985). Moreover, since the B lymphocytes that they possess are immature, the humoral immune system of Xid mice is functionally impaired (Scher et al. 1975a).

Although the B lymphocytes of Xid mice phenotypically resemble the immature B lymphocytes of normal mice (Lyb3-, Lyb5-, Lyb7-, high surface IgM/IgD) (Scher 1982b), there are a number of important differences. These include the ability to respond to phenol-extracted lipopolysaccharide (Ono et al. 1983), expression of minor lymphocyte stimulating (Mls) determinants (Webb et al. 1984), migration through lymphoid tissues (Sprent et al. 1985) and long-term survival in double bone marrow, irradiation chimeras (Sprent and Bruce 1984). These differences indicate that Xid B cells represent a distinct and abnormal population that is not present in normal mice.

The apparent block in the differentiation pathway of B cells in Xid mice is not absolute. It has been shown that the Peyer's patches of young, adult Xid mice contain B cells with a mature phenotype (Lyb5+, low surface IgM density), and that these cells are capable of responding, in vitro, to the type II T-independent antigen, TNP-Ficoll (Eldridge et al. 1983; 1984). Furthermore, splenic B cells from Xid mice can be forced to differentiate in vitro, into TNP-Ficoll-reactive plaque-forming cells, under the influence of helper T cells and accessory cells from Peyer's

patches of Xid or normal mice (Eldridge, Beagley and McGhee 1987). Moreover, spleen cells from one year old Xid mice are capable of responding to TNP-Ficoll, in vitro (Fidler, Morgan and Weigle 1980). These results indicate that the maturation of B cells in Xid mice is not completely blocked, but that the defects vary between tissues and are partially corrected with age.

Lyl+ B cells represent a distinct lineage of self-renewing B cells that are present at high frequency in the peritoneal cavity of normal, adult mice (Herzenberg et al. 1986). These cells are absent from adult mouse bone marrow and lymph nodes, and constitute less than 2% of B cells in adult mouse spleen (Herzenberg et al. 1986). Lyl+ B cells fall into the category of Lyb5+ B cells (Smith et al. 1985), and are absent in Xid mice (Hayakawa, Hardy and Herzenberg 1986; Herzenberg et al. 1986). However, Xid mice contain precursors for Lyl+ B cells (De la Hera et al. 1987). Thus, there appears to be a block in the differentiation pathway of Lyl+ B cells, as well.

The defective nature of B lymphocytes in Xid mice results in the selective impairment of certain humoral immune responses.

1.3.1 Impairment of Certain Humoral Immune Responses in Xid Mice

One of the definitive immunodeficiencies of Xid mice is their inability to mount antibody responses to type II T-independent antigens, such as type III pneumococcal polysaccharide (Amsbaugh et al. 1972), TNP-Ficoll (Boswell et al. 1980), polyinosinic-polycytidylic acid (poly-I-C) (Scher, Frantz and Steinberg 1973) and phosphoryl-choline-bacteriophage

T4 (PC-T4) (Quintans and Kaplan 1978). These antigens are thought to stimulate the mature subset of conventional B lymphocytes in normal mice (Ono et al. 1983). Thus, the absence of this cell subset in Xid mice (Scher 1982b) may account for the non-responsiveness to type II T-independent antigens observed in this strain.

The antibody responses of Xid mice to type I T-independent antigens, such as TNP-Brucella abortus and TNP-lipopolysaccharide, are equivalent to those of normal mice (Boswell et al. 1980).

B lymphocytes from Xid mice make defective proliferative responses to the B cell mitogens lipopolysaccharide (LPS) (Scher et al. 1975a; Rosenstreich et al. 1978; Ono et al. 1983), poly I-C (Scher et al. 1975a) and anti-IgM antibody (Sieckmann et al. 1978a; 1978b).

The responses of Xid mice to T-dependent antigens are selectively defective. These mice made deficient primary antibody responses to sheep red blood cells (SRBC) and TNP-keyhole limpet hemocyanin (TNP-KLH) (Scher, Berning and Asofsky 1979; Boswell et al. 1980). These deficiencies were not due to defective helper T cell or antigen-presenting cell functions. Helper T cells and antigen-presenting cells from Xid mice were effective at stimulating an antigen-dependent response by B cells from normal mice in vitro. However, B cells from Xid mice were not stimulated by the combination of antigen, helper T cells and antigen-presenting cells from normal mice. In contrast to their defective primary antibody responses to SRBC and TNP-KLH, Xid mice made quantitatively normal secondary antibody responses after being boosted with these antigens (Scher, Berning and Asofsky 1979; Stein et al. 1980; Scher 1982a).

Xid mice also made defective primary antibody responses to the T-dependent antigen phosphorylcholine-KLH (PC-KLH) (Quintans and Kaplan 1978; Kenny et al. 1981). Upon secondary immunization with this antigen, the antibody responses of Xid mice improved, but were still lower than those of normal mice (Kenny et al. 1981). In addition, the secondary antibody response to PC-KLH of Xid mice was altered with respect to idiotype and isotype. Whereas normal mice made IgM antibodies and antibodies of the T15 idiotype, Xid mice failed to make this class of antibody, and few of their antibodies were of the T15 idiotype. These results indicate that Xid mice have both isotype and idiotype defects in their antibody responses to certain T-dependent antigens.

The IgM and IgG3 isotypes are particularly defective in Xid mice. The levels of total serum IgM in Xid mice are approximately 30% of those in normal mice, and their levels of serum IgG3 are less than 10% (Perlmutter et al. 1979; Eldridge, Meulbroek and McGhee 1988). Other IgG isotypes are present in Xid sera at relatively normal levels. Elevated levels of IgA are present in the sera of Xid mice, compared to normal mice, but the levels of secretory IgA in saliva, respiratory and gastrointestinal secretions are similar in Xid and normal mice (Eldridge, Meulbroek and McGhee 1988). Oral administration of the T-dependent antigen, SRBC, evoked Peyer's patch IgA responses in Xid and normal mice that were quantitatively similar (Kiyono et al. 1983).

1.3.2 T cells and Accessory Cells of Xid Mice

The T cell functions of Xid mice are essentially intact. Xid T cells made normal proliferative responses to T cell mitogens (concanavalin A and phytohemagglutinin) and killed allogeneic tumour cells in a normal manner (Scher et al. 1975a). In addition, Xid mice rejected skin allografts as rapidly as normal mice (Scher et al. 1975a). The results of several studies have indicated that Xid T cells provide adequate help for T-dependent antibody responses by normal B cells (Janeway and Barthold 1975; Scher, Berning and Asofsky 1979; Boswell et al. 1980; Letvin, Huber and Benacerraf 1982). Only two subtle defects in Xid T cell function have been reported: (1) unlike normal T cells, Xid T cells were unable to augment LPS-stimulated, polyclonal B cell responses (Goodman and Weigle 1979), and (2) unlike normal T cells, Xid T cells failed to proliferate in response to a cloned dendritic cell line (Clayberger et al. 1985).

There is no evidence that abnormal levels of T cell-mediated suppression are responsible for the observed immunodeficiencies in Xid mice. In several systems, the presence of Xid cells did not suppress the immune responses of cells from normal mice (Quintans and Kaplan 1978; Scher, Berning and Asofsky 1979; Goodman and Weigle 1979; Ono et al. 1983; Clayburger et al. 1985).

The antigen presentation function of accessory cells from Xid mice is not entirely equivalent to that of accessory cells from normal mice. While splenic adherent cells (SACs) from Xid and normal mice were equally effective at supporting an antibody response by normal B cells to

TNP-KLH (Boswell et al. 1980), Xid SACs, pulsed with beef insulin failed to induce proliferation of insulin-specific, histocompatible T cells (Rosenwasser and Huber 1981). In addition, Xid SACs were less efficient than normal SACs in supporting an antibody response by normal B cells to TNP-Ficoll (Letvin, Huber and Benacerraf 1982). Thus, there are some antigen presentation defects in Xid mice, and these appear to depend on the antigen in question.

Macrophages from Xid mice produced normal amounts of prostaglandins and lymphocyte-activating factor (LAF) in response to stimulation with LPS (Rosenstreich et al. 1978). In addition, Xid and normal mice were equally able to control the early phase of Salmonella typhimurium infection; this resistance is due to the ability of macrophages to restrict bacterial replication (O'Brien, Scher and Metcalf 1981). Thus, in these functions, Xid macrophages appear to be normal.

1.3.3 Infectious Diseases in Xid Mice

The response of Xid mice to infectious disease has been studied with a number of systems. In some cases the Xid defect conferred greater susceptibility to infection, and this was accompanied by deficient antibody production. For example, Salmonella typhimurium is particularly pathogenic in Xid mice; the LD50 value in this strain was 1000-fold lower than in resistant, normal mice (O'Brien et al. 1979). While Xid mice were able to limit the early phase of this infection and mount normal delayed-type hypersensitivity reactions to the bacteria, they made delayed and deficient antibody responses (O'Brien, Scher and Metcalf 1981). Xid mice

generated few Salmonella-reactive B cells, and these had an altered isotype profile and epitope-specificity pattern relative to normal mice (Duran and Metcalf 1987). Passive transfer of antibody from normal mice increased the resistance of Xid mice to S. typhimurium infection (O'Brien, Scher and Metcalf 1981). These results indicate that the humoral immune defect in Xid mice renders them susceptible to S. typhimurium infection.

Xid mice made defective antibody responses to immunization with another gram-negative bacteria, Pseudomonas aeruginosa (Zweerink et al. 1988). Xid and normal mice were equally resistant to infection with this organism, but Xid mice, rendered neutropenic by treatment with cyclophosphamide, were 1000 times more susceptible than similarly treated, normal mice. Passive transfer of monoclonal anti-P. aeruginosa antibody restored resistance in neutropenic Xid mice. These results demonstrate the contribution of antibody in resistance to gram-negative bacteria, but further illustrate the importance of other immune elements in resistance to this disease.

Xid mice made defective antibody responses to immunization with formalin-inactivated, influenza virus vaccines, but were nevertheless protected against subsequent lethal challenge with live virus (Lucas, Barry and Kind 1978; Reale, Bona and Schulman 1985). Since resistance to this infection is known to correlate with levels of circulating antibody (Kaye, Dowdle and McQueen 1969), these results imply that Xid mice were able to make the correct isotypes and specificities of antibodies required for protection.

Xid mice are more susceptible to malaria caused by the mouse parasite Plasmodium yoelii (Hunter et al. 1979). These mice had higher

and more prolonged parasitemia than normal mice, but eventually eliminated the infection and were subsequently resistant to reinfection (Jayawardena, Janeway and Kemp 1979). During the primary infection with P. yoelii, Xid mice made a delayed serum IgM antibody response which was temporally associated with the eventual elimination of the parasite. B cells from immune, Xid mice were less efficient than B cells from immune, normal mice in transferring resistance to naive recipients. However, T cells from immune, Xid mice plus B cells from immune, normal mice were as effective as T cells plus B cells from immune, normal mice. The results of this study indicated a role for B cells and antibody in resistance to P. yoelii infection, but suggested that T cells are also crucial for elimination of this parasite.

Upon immunization with irradiated cercariae of the parasite, Schistosoma mansoni, Xid mice made defective IgM antibody responses, but normal IgG antibody responses (Correa-Oliveira and Sher 1985). Nevertheless, vaccinated, Xid and normal mice were equally, though only partially, protected against a subsequent challenge infection with the live parasite. Similarly, Xid mice made defective IgM and IgG antibody responses to infection with Toxoplasma gondii, but were not more susceptible to infection with this parasite (Brinkmann, Remington and Sharma 1987). These results indicate that other immune elements, which are intact in Xid mice, mediate immunity to these parasites.

Increased susceptibility to a particular infection in Xid mice, relative to normal mice, suggests that B cells and antibodies are important in immunity to that infection. However, such evidence should be supported by the demonstration of defective antibody responses in Xid

mice, and is strengthened by the demonstration of increased resistance conferred by the transfer of B cells or antibody. Such studies do not rule out the contribution of other immune elements in immunity to the infection.

1.3.4 Summary

The CBA/N mouse bears an X-linked immunodeficiency gene, the expression of which results in defective B cell maturation with the consequent impairment of certain humoral immune responses. The antibody responses of these mice to T-dependent antigens are quantitatively diminished and have altered isotype and idiotype profiles. Some antigens are not recognized at all by CBA/N mice. The T cell functions of CBA/N mice are essentially intact. However, there may be some selective antigen presentation defects in CBA/N mice.

1.4 Giardiasis in CBA/N Mice

CBA/N mice develop chronic infections when infected with the parasite, G. muris (Snider, Skea and Underdown 1988). Most immunocompetent strains of mice eliminate this parasite within 5 to 10 weeks of infection (Belosevic et al. 1984). Breeding studies and studies using congenic mice demonstrated that susceptibility to chronic giardiasis was associated with expression of the Xid gene (Snider, Skea and Underdown 1988). Xid mice made quantitatively normal or elevated serum and intestinal IgA antibody responses to G. muris infection. However, the serum IgG antibody response to infection was lower in Xid mice, compared to that in normal mice.

1.4.1 Hypotheses Concerning the Basis for Chronic Giardiasis in CBA/N Mice

Since CBA/N mice have a prominent defect in B cells and humoral immunity (Scher 1982a), but intact T cell functions (Scher et al. 1975a), I hypothesized that susceptibility to chronic giardiasis in this strain was due to the B cell defect. This hypothesis was tested by adoptively transferring cells from immunocompetent, histocompatible, CBA/Ca mice to

CBA/N mice, in an attempt to reconstitute the ability of CBA/N mice to eliminate G. muris.

Assuming that the defect of CBA/N mice that renders them susceptible to chronic giardiasis is the B cell defect, this may be manifested in either an isotype defect or a specificity defect. That is, CBA/N mice may fail to synthesize the correct class of antibody required for elimination of the parasite, or CBA/N mice may fail to recognize the crucial antigen or antigens on the parasite that stimulate immune elimination in other strains of mice. Since CBA/N mice made a quantitatively deficient serum IgG antibody response to G. muris infection (Snider, Skea and Underdown 1988), I considered the hypothesis that serum IgG antibody may participate in elimination of the parasite. This hypothesis was tested two ways: (1) by parenterally immunizing CBA/N mice with G. muris trophozoites to boost circulating levels of serum IgG anti-G. muris antibody, and (2) by passively transferring hyperimmune anti-G. muris antiserum from CBA/J mice to CBA/N mice. In these experiments, the influence of serum IgG antibody on elimination of G. muris was tested.

Although CBA/N mice made quantitatively normal or elevated IgA antibody responses to infection with G. muris (Snider, Skea and Underdown 1988), it is possible that these responses failed to include antibodies directed toward the crucial antigens on the parasite. This hypothesis was tested by examining the antigen recognition profiles of antibodies from CBA/N mice and CBA/Ca mice. I used the technique of Western blotting to examine protein and glycoprotein antigens of the parasite, and organic solvent extraction, together with dot blot analysis and thin-layer chromatography, to examine lipid and glycolipid antigens. I looked for

an antigen that was recognized by antibodies from CBA/Ca mice, but not by antibodies from CBA/N mice.

1.4.2 Resistance to Reinfection with G. muris

Since it is known that the immune mechanisms that mediate elimination of a primary infection are not necessarily the same as those that mediate resistance to reinfection (Mitchell, McMichael and Lamb 1985), I examined the resistance of CBA/N mice to reinfection with G. muris, following drug-cure of a primary infection. In addition, I examined the ability of cells from G. muris-immune mice to transfer resistance to the infection to naive recipient mice. Possible differences between the immune mechanisms that mediate elimination of a primary infection and those that mediate protection against reinfection have important implications for the development of effective vaccines.

Chapter 2

METHODS AND MATERIALS

2.1 Mice

Male CBA/NJ, CBA/CaJ, CBA/J and BALB/cJ mice at 5-6 weeks of age were purchased from Jackson Laboratories (Bar Harbor, ME). Mice were housed at the Central Animal Facility in the Health Sciences Centre at McMaster University. Mice were maintained on standard rodent chow and water, ad libitum. Their drinking water was supplemented with 8.8 mg/mL piperazine ("Ascapel" - M.T.C. Pharmaceuticals, Cambridge, ON) to avoid helminth infections. All mice were 6-10 weeks of age at the beginning of experiments.

Female nu/nu (CD-1) BR (nude) mice were purchased from Charles River Laboratories (St. Constant, PQ). Nude mice were housed in sterile cages with filter hoods and maintained on autoclaved bedding, chow and water. G. muris was perpetuated in nude mice. The mice were infected at 6-8 weeks of age and the infection was transferred to new mice within 6 weeks.

2.2 Infection of Mice with G. muris

G. muris cysts were isolated from the feces of infected nude mice, as described (see 2.3). A suspension of 5000 cysts in 0.2 mL of saline (0.15M NaCl) was administered to each mouse, per os, using a blunt,

curved, 16-gauge feeding needle (Popper and Sons, Inc., New Hyde Park, NY).

2.3 Isolation and Counting of G. muris Cysts

The course of G. muris infection in mice was followed by measuring cyst output in feces. Cysts were isolated and counted as previously described (Roberts-Thomson et al. 1976a). Briefly, mice were placed in clean plastic containers and 8 fresh fecal pellets were collected. The pellets were incubated in saline (4 pellets per 2.5 mL saline) for 0.5 hours at room temperature, following which the pellets were broken up in the saline using wooden applicator sticks (Fisher Scientific, Toronto, ON). The resulting suspensions were layered onto 2.5 mL of 1M sucrose (BDH Chemicals, Toronto, ON) and centrifuged at 300 x g for 10 minutes at 4°C. The interface material (approximately 1.5 ml) was removed and mixed with 3 mL of saline. These suspensions were centrifuged at 600 x g for 5 minutes at 4°C. The pelleted cysts were resuspended in 3 mL of saline and centrifuged at 600 x g for 2 minutes at 4°C. The repelleted cysts were resuspended in 1 mL of saline (cysts in the two tubes representing one mouse were combined) and counted using a hemocytometer. The limit of detection was 600 cysts from 8 fecal pellets.

2.4 Drug Treatment of G. muris-infected Mice

In some experiments mice were cured of their G. muris infections by treatment with metronidazole (Poulenc Ltd., Montreal, PQ). Mice were treated on 3 consecutive days with 0.4 mL of 12 mg/ml metronidazole in saline. The drug was administered, per os, using a blunt, curved 16-gauge

feeding needle (Popper and Sons, Inc., New Hyde Park, NY). One week after the last treatment, feces from the treated mice were screened for the presence of cysts. No cysts were detected in the feces of cured mice.

2.5 Isolation of G. muris Trophozoites

Trophozoites were isolated from the small intestines of infected nude mice as previously described (Nair, Gillon and Ferguson 1981). Briefly, mice were sacrificed by cervical dislocation and two 9 cm lengths of small intestine proximal to the jejunal end were excised and everted over spiral metal rods. The everted intestinal segments were vibrated (Vibromixer El, Chemap, Zurich, Switzerland) at high speed for 3 minutes into 50 mL of phosphate buffered saline (PBS - 0.01 M phosphate, 0.15 M NaCl, pH 7.4), on ice. The mixture was filtered through gauze and centrifuged at 30 x g for 2 minutes at 4°C to remove epithelial cell clumps and debris. The trophozoites were then washed twice by centrifugation at 400 x g for 5 minutes at 4°C. The pelleted trophozoites were resuspended in 1 mL of PBS and counted using a hemocytometer.

2.6 Collection of Sera

Mice under deep ether anesthesia were bled from the axillary vein. Anesthetized mice were placed on their backs and their forepaws were extended and restrained. A deep cut through the axilla was made using a No. 20 scalpel blade. Blood collecting in the axillary fossa was removed using a 14.5 cm pasteur pipette. Following this procedure, mice were sacrificed by cervical dislocation.

Alternatively, mice were bled from the orbital sinus using a 23 cm pasteur pipette, as previously described (Cunliffe-Beamer 1983). Mice recovered from this procedure.

Blood was allowed to clot for 1 hour at room temperature, then overnight at 4°C. Sera were removed and centrifuged at 1200 x g for 10 minutes at 4°C to remove any remaining cells. Sera were stored at -20°C until use.

2.7 Adoptive Transfer of Cells Between Mice

2.7.1 Cell Suspensions

Spleen cell suspensions were prepared, as previously described (Goding 1983), in RPMI 1640 supplemented with 10% fetal bovine serum (FBS - Gibco Laboratories, Grand Island, NY - heat inactivated for 1 hour at 56°C). Peyer's patch and mesenteric lymph node cell suspensions were prepared as previously described (McDermott and Bienenstock 1979) in the same media. All cell suspensions were washed twice by centrifugation at 300 x g for 10 minutes at 4°C prior to use.

2.7.2 T cell Depletion

Spleen cell populations were enriched for B cells by specifically depleting T cells using anti-Thy 1.2 antibody and complement (MacDonald 1987). Cells were treated with tissue culture supernatant derived from the IgM anti-Thy 1.2 antibody-producing hybridoma HO-13-4 (Marshak-Rothstein et al. 1979). The cells were incubated at a concentration of 40 X 10^6 cells per 10 mL of 1:5 HO-13-4 supernatant, on ice, for 60

minutes. The treated cells were centrifuged at 300 x g for 10 minutes at 4°C, following which they were resuspended in media containing the complement system. The cells were incubated at a concentration of 50 X 10^6 cells per 1 mL of 1:10 Low Tox-M Rabbit Complement (Cedarlane Laboratories, Ltd., Hornby, ON) in media, for 45 minutes at 37°C. The cells were centrifuged as before and then washed twice by centrifugation. The cells surviving the treatment were counted using a hemocytometer, and their viability was determined by trypan blue exclusion (Mishell et al. 1980).

2.7.3 B cell Depletion

Spleen cell populations were enriched for T cells by specifically depleting B cells by panning on petri dishes coated with anti-immunoglobulin (Ig) antibody (Wysocki and Sato 1978). Affinity purified sheep anti-mouse Ig antibody was prepared in our laboratory by standard techniques (Cuatrecasas and Anfinson 1971). Ouchterlony analysis demonstrated reactivity of this antibody against mouse IgM, IgG, IgA and isolated κ chains (data not shown). Bacteriological petri dishes (100 X 15 mm -- Fisher Scientific, Toronto, ON) were each treated with 10 mL of 10 μg/mL of this antibody in 0.05 M Tris-HCl, pH 9.5, for 40 minutes at room temperature. The dishes were rinsed 3 times with 5 mL of PBS, then once with 5 mL of PBS containing 1% FBS. A total of 24 X 10^6 cells in 3 mL of media were added to each anti-Ig antibody-coated plate. The panning incubation was conducted for a total of 70 minutes; the dishes were swirled once after 40 minutes. At the end of the incubation, the cells were pipetted from the dishes. The dishes were rinsed gently with 5 mL

of media, and this was added to the cells. The cells were centrifuged at 300 x g for 10 minutes at 4°C, and then washed twice by centrifugation. These cells, that were non-adherent on the anti-Ig antibody-coated plates, were counted using a hemocytometer, and their viability was determined by trypan blue exclusion (Mishell et al. 1980).

2.7.4 Monitoring of T cell and B cell Depletions

2.7.4.1 F.A.C.S.

Whole spleen cell populations, T cell-depleted populations and B cell-depleted populations were analyzed for the presence and proportion of T cells and B cells using a fluorescence-activated cell sorter (FACS). Fluorescein isothiocyanate - (FITC-) conjugated anti-Thy 1.2 antibody (NEN - Dupont Canada, Mississauga, ON) was used to identify T cells, and FITC-anti Ig antibody (Cedarlane Laboratories, Ltd., Hornby, ON) was used to identify B cells. Flow cytometry was performed by B. Kurc of McMaster University using the Coulter Epics V System (Coulter Electronics, Inc., Hialeah, FL). Results of one experiment are provided (Table 1).

2.7.4.2 Mitogenic Stimulation of Cells

T cell and B cell depletions were also monitored by measuring the proliferation of cells stimulated with mitogens (Bradley 1980). The T cell mitogen Concanavalin A (Con A - Pharmacia (Canada) Inc., Baie D'Urfe, PQ), and the B cell mitogen, lipopolysaccharide (E. coli, serotype OSS-B5 (LPS) - Sigma Chemical Co., St. Louis, MO), were used. Triplicate wells of a 96-well tissue culture plate (Falcon - Becton-Dickinson, Lincoln

TABLE 1

T CELL AND B CELL DEPLETIONS FROM SPLEEN CELL SUSPENSIONS

CELL POPULATION	FACS ANALYSIS		MITOGEN STIMULATION INDEX	
	PERCENT Thy1.2+	PERCENT Ig+	CON.A	LPS
CBA/Ca				
Whole spleen	37.3	44.0	3.9	5.7
B cells (T-depleted)	2.6	81.1	1.1	6.4
T cells (B-depleted)	77.8	3.9	3.1	1.4
CBA/N				
Whole spleen	54.5	36.6	2.4	1.7
B cells (T-depleted)	6.3	71.2	1.2	1.7
T cells (B-depleted)	78.8	4.8	3.9	1.3

Spleen cell suspensions from CBA/Ca mice and CBA/N mice were treated with anti-Thy1.2 antibody and complement to deplete T cells, or were subjected to panning on anti-immunoglobulin antibody-coated petri dishes to deplete B cells. The whole spleen cell suspensions and the T cell-depleted and B cell-depleted suspensions were analyzed by FACS, using FITC-anti Thy1.2 antibody to identify T cells (Thy1.2+), and anti-immunoglubulin antibody to identify B cells (Ig+). These data represent a single analysis of cell suspensions used in one experiment. In addition, the cell suspensions were stimulated with the T cell mitogen, concanavalin A (Con.A) or with the B cell mitogen lipopolysaccharide (LPS). These data represent the values obtained by dividing the average cpm incorporated by stimulated cells in triplicate wells by the average cpm incorporated by unstimulated cells in triplicate wells.

Park, NJ) were set up, each containing 0.5 X 10^6 cells in 0.1 mL of RPMI 1640 supplemented with 10% FBS. Cultures were stimulated with 0.1 mL of 10 μg/mL LPS, or with 0.1 mL of 2 μg/mL Con A. Control cultures received 0.1 mL of media. Cultures were incubated for 24 hours at 37°C in a humidified atmosphere containing 5% CO_2. Following this, 1 μCurie of ^{3}H-thymidine (ICN Biomedicals, Montreal, PQ) was added to each well, and the incubation was continued for a further 24 hours. Cultures were terminated by freezing at -20°C. After thawing, cells were harvested using a PHD Cell Harvester and glass fiber filters (Cambridge Technology Inc., Watertown, MA). Filters were dried at room temperature, overnight, and placed in scintillation vials with 2.5 mL of aqueous counting scintillant (Amersham Canada Ltd., Oakville, ON). The amount of ^{3}H-thymidine on the filters was measured using an LKB Wallac 1209 Rackbeta Liquid Scintillation Counter. Results were expressed as the stimulation index, that is, the average counts per minute (cpm) incorporated by cells in the stimulated wells divided by the average cpm incorporated by cells in the control wells. Results of one experiment are provided (Table 1).

2.7.5. Reconstitution of Mice

For reconstitution of mice, cell suspensions were adjusted to a concentration of 250 X 10^6 (viable) cells per mL. A volume of 0.2 mL, containing 50 X 10^6 (viable) cells, was injected into the tail vein of each recipient mouse. Mice were infected with *G. muris* 3 weeks after reconstitution. The course of infection in the reconstituted mice, and in intact CBA/N and CBA/Ca control mice, was monitored by measuring cyst output in feces, as described (see 2.3).

In some experiments, mice received a lethal dose of radiation prior to reconstitution. The radiation source was ^{137}Cs. The parameters of the irradiation procedure were calibrated by S. Staniek of McMaster University using a Farmer ion-chamber, pre-calibrated for ^{137}Cs by the Ontario Ministry of Labour. Mice were placed in the peripheral compartments of a circular restraining device. The device was placed under the source and was rotated throughout the exposure. Under the pre-set conditions, an exposure of 58 minutes and 26 seconds resulted in a lethal dose of 1000 rads.

2.8 Immunization of Mice with G. muris Trophozoites

Trophozoites were isolated from the intestines of infected nude mice, as described (see 2.5). The suspension was adjusted to a concentration of 2.5 X 10^6 trophozoites per mL of PBS, and was mixed with an equal volume of Freund's Complete Adjuvant (FCA-Difco Laboratories, Detroit, MI). A stable emulsion was formed by rapidly passing the mixture back and forth between two glass syringes joined by a stainless steel connector. A volume of 0.4 mL of emulsion, containing 0.5 X 10^6 trophozoite equivalents, was injected into each mouse, intraperitoneally. Sham-immunized mice received 0.4 mL of a stable emulsion formed by mixing equal volumes of PBS and FCA. The immunization was repeated 30 days later.

2.9 Passive Transfer of anti-G. muris Antisera to CBA/N Mice

Hyperimmune anti-G. muris antisera were produced by immunization of CBA/J mice and CBA/N mice, as described (see 2.8). Mice were bled from

the orbital sinus, 8, 18 and 30 days after the second immunization, and were terminally bled from the axillary vein, 8 days after the last orbital bleed. The sera were pooled according to strain prior to use. The relative amounts of IgG anti-G. muris antibody in the CBA/J and CBA/N antisera were determined by immunoradiometric assay (see 2.12). The CBA/J antiserum contained approximately 3 times as much IgG anti-G. muris antibody (10,000 arbitrary antibody units per mL) as the CBA/N antiserum (3,450 arbitrary antibody units per mL).

The CBA/J and CBA/N antisera were injected, intraperitoneally, into groups of CBA/N mice infected with G. muris. Since the half-life of mouse IgG is 4.5 days (Waldmann and Strober 1969), the injections were repeated every 5 days, beginning 12 days prior to infection and continuing throughout the infection. A total of 20 injections of 0.2 mL of serum, each, were administered to each mouse over a period of 95 days. Two days after the last injection (12 weeks post-infection) mice were terminally bled from the axillary vein. The presence of passively transferred antibody in the sera of the recipient mice was confirmed by immunoradiometric assay.

2.10 Enzyme-Linked Immunosorbent Assay for Total Serum IgM

A "sandwich" type enzyme-linked immunosorbent assay (ELISA) was used to measure total serum IgM levels.

2.10.1 Coating Antibody

Rabbit anti-mouse IgM antibody was purified by affinity chromatography (Cuatrecasas and Anfinson 1971) on a column consisting of a mouse

monoclonal IgM λ (MOPC 104) coupled to Sepharose 4B (Pharmacia (Canada) Inc., Baie D'Urfe, PQ). Alternate rows of wells in a 96-(flat bottom) well, polystyrene, microtitre plate (Nunc - Gibco Canada Inc., Burlington, ON) were coated with 0.05 mL of 10 μg/mL affinity purified antibody in PBS, overnight at 4°C. The remainder of the wells were left blank. Unbound sites on all of the wells were blocked by incubation with 5% normal goat serum (ICN Biomedicals, Montreal, PQ) in PBS (NGS-PBS) for 1 hour at room temperature. The wells were then washed 5 times with PBS containing 0.05% Tween-20 (PBS-Tw).

2.10.2 Assay Procedure

Serum samples were diluted 1:5000 with NGS-PBS, and 0.05 mL aliquots of the dilute samples were added to duplicate antibody-coated wells and duplicate blank wells. After a 3 hour incubation at room temperature, the wells were emptied and washed 5 times with PBS-Tw. Goat anti-mouse IgM antibody conjugated to horse radish peroxidase (GaMIgM-HRP - (KPL) - Mandel Scientific Co., Rockwood, ON) was diluted 1:500 with NGS-PBS, and 0.05 mL of the dilute conjugate was incubated in each well for 3 hours at room temperature. The wells were then emptied and washed 5 times with PBS-Tw. A substrate solution (Hudson and Hay 1980) consisting of 0.05 mL of 0.4 mg/ml o-phenylenediamine (Sigma Chemicals, St. Louis, MO) and 0.015% hydrogen peroxide (Caledon Laboratories, Georgetown, ON) in 0.1M phosphate-0.05M citrate buffer, pH 5.0, was added to each well. Colour development was allowed to proceed for 15 minutes at room temperature, in the dark. The reaction was terminated by the addition of 0.05 mL of 2N H_2SO_4 to each well. The optical density at 490 nm in each

well was measured using a Dynatech MR600 Microplate Reader. The average optical density in the duplicate blank wells was subtracted from the average optical density in the duplicate antibody-coated wells to obtain the "Δ O.D. (490 nm)" result.

2.10.3 Specificity

The isotype specificity of the GaMIgM-HRP reagent was tested by a direct ELISA using purified monoclonal immunoglobulins (IgG1κ, IgG3κ, IgAκ, IgAλ, IgMκ and IgMλ - purified in our laboratory by I. Switzer using affinity chromatography and high pressure liquid chromatography techniques) and isolated κ and λ chains (Bence-Jones proteins - purchased from Mandel Scientific Co., Rockwood, ON). Wells of microtitre plates were coated with 0.05 mL of 10 μg/mL of the pure proteins and were blocked and washed as described above. The GaMIgM-HRP was diluted 1:500 with NGS-PBS and 0.05 mL of the dilute conjugate was incubated in each of the wells for 3 hours at room temperature. The wells were washed and colour development was performed as described above (see 2.10.2). Under these conditions, the GaMIgM-HRP reacted with only the IgMκ and IgMλ proteins (Δ O.D. (490 nm) >1.9), and not with any of the other proteins (Δ O.D. (490 nm) <0.1).

In addition, the ELISA for total serum IgM was performed using the pure proteins at a concentration of 0.06 μg/mL in place of the serum samples. Under these conditions, only the IgMκ and IgMλ proteins gave a positive reaction (Δ O.D. (490 nm) >1.5), while the other proteins gave a negative reaction (Δ O.D. (490 nm) <0.1).

2.10.4 Standard Curve and Determination of Serum IgM Levels

A standard curve for the ELISA for total serum IgM was developed using a mouse immunoglobulin reference serum (ICN Biomedicals, Montreal, PQ) containing 227 μg/mL IgM. A standard curve was constructed for each group of sera assayed. A sigmoidal curve was fit to the standard curve by a computer program (Davis et al. 1980), adapted to BASIC programming by M. Schiff of the University of Toronto. An example from one experiment is provided (Figure 1). The concentration of IgM, in μg/mL, in a serum sample was computed from its Δ.O.D. (490) result, by interpolation of the fitted standard curve.

2.11 Analysis of the Antibody Response to a Type-II T-Independent Antigen

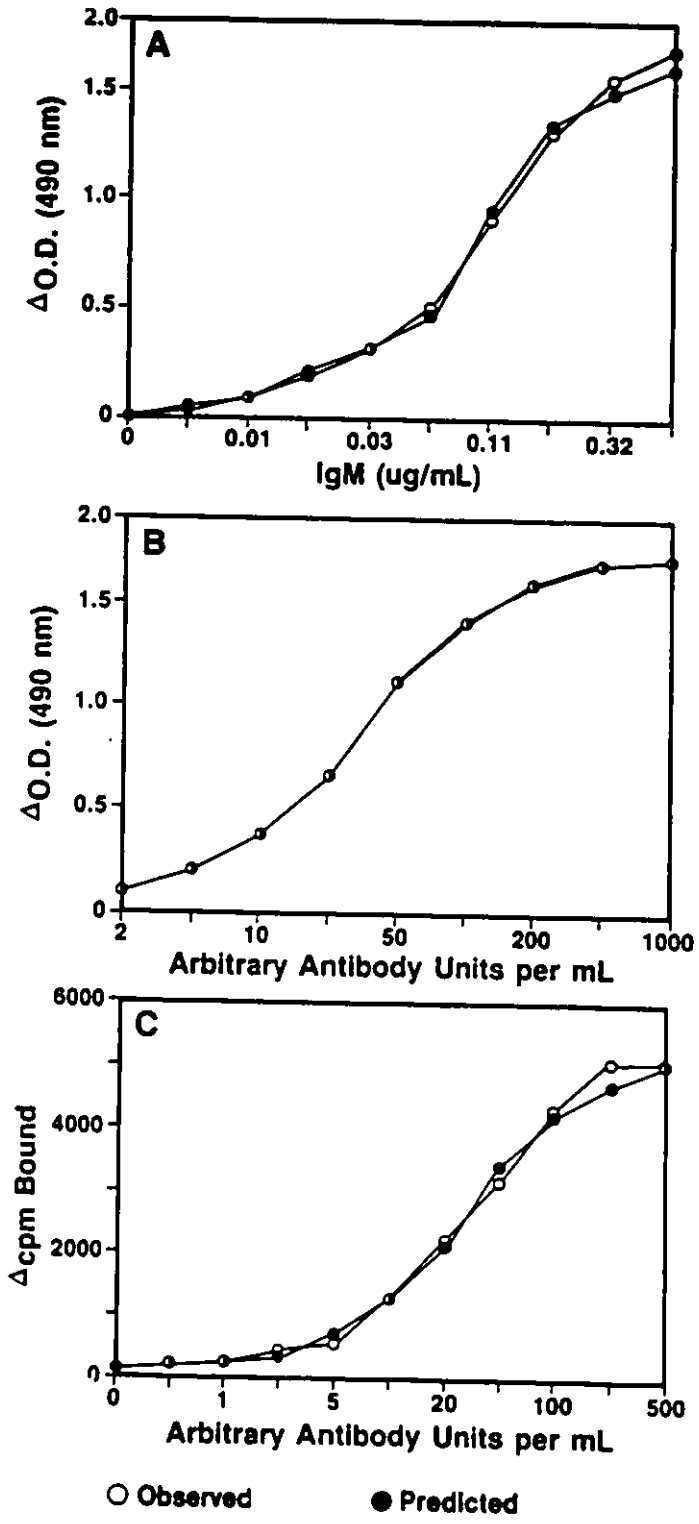
The type II T-independent antigen, trinitrophenyl Ficoll (TNP_{187}-Ficoll) (Eldridge, Beagley and McGhee 1987) was a gift from J. Eldridge of the University of Alabama. After drug cure of G. muris infection (see 2.4), mice were immunized by intraperitoneal injection of 100 μg of TNP_{187}-Ficoll in 0.2 mL of PBS. Fifteen days later, the mice were terminally bled from the axillary vein, and their serum antibody response to the immunization was measured by ELISA.

2.11.1 ELISA

The coating antigen for this ELISA was the cross-reacting antigen, dinitrophenyl-bovine gamma globulin (DNP-BGG). This conjugate, prepared as previously described (Hodes and Singer 1975), was used so that the T-cell independent, hapten-specific response could be measured.

Figure 1

Standard curves were generated for (A) the ELISA for total serum IgM, (B) the ELISA for anti-TNP antibody and (C) the IRMA for IgG anti-*G. muris* antibody. A computer program was used to predict a mathematical curve (●) for each of the observed curves (O), and to calculate IgM and antibody concentrations from given values of ΔO.D. (490 nm) and Δ cpm bound.


A
2.0
1.5
1.0
0.5
0
ΔO.D. (490 nm)
0
0.01
0.03
0.11
0.32
IgM (ug/mL)
B
2.0
1.5
1.0
0.5
0
ΔO.D. (490 nm)
2
10
50
200
1000
Arbitrary Antibody Units per mL
C
6000
4000
2000
0
Δcpm Bound
0
1
5
20
100
500
Arbitrary Antibody Units per mL
○ Observed
● Predicted

Alternate rows of wells in microtitre plates (Nunc-Gibco Canada, Burlington, ON) were coated with 0.05 mL of 10 μg/mL DNP-BGG in PBS, overnight at 4°C. The remainder of the wells were left blank. Unbound sites on all of the wells were blocked by incubation with NGS-PBS for 1 hour at room temperature. The wells were emptied and washed 5 times with PBS-Tw. Serum samples were diluted 1:100 with NGS-PBS, and 0.05 mL aliquots of the dilute samples were added to duplicate antigen-coated wells and duplicate blank wells. After a 3 hour incubation at room temperature, the wells were emptied and washed 5 times with PBS-Tw. Goat anti-mouse Ig-horse radish peroxidase (GaMIg-HRP (KPL), Mandel Scientific Co., Rockwood, ON) was diluted 1:500 with NGS-PBS and 0.05 mL of the dilute conjugate was added to each well. The GaMIg-HRP reagent was shown by direct ELISA (see 2.10.3) to react with IgG, IgM, IgA and isolated κ chains (Δ.O.D. (490 nm) > 1.9). After a 3 hour incubation at room temperature, the wells were emptied and washed 5 times with PBS-Tw. Colour development was performed as described (see 2.10.2).

A standard curve of antibody activity was constructed using serum from one TNP_{187}-Ficoll-immunized CBA/Ca mouse. This serum was assigned a value of 10,000 arbitrary antibody units per mL. A standard curve was prepared for each group of sera assayed. A sigmoidal curve was fit to the standard as described (see 2.10.4). An example from one experiment is provided (Figure 1). The amount of antibody activity, in arbitrary antibody units/mL, in a serum sample was computed from its Δ.O.D. (490 nm) result, by interpolation of the fitted standard curve.

2.12 Immunoradiometric Assay for IgG anti-G. muris Antibody

A solid-phase immunoradiometric assay (IRMA) was used to measure IgG anti-*G. muris* antibody in sera.

2.12.1 Coating Antigen

Trophozoites, isolated from the intestines of infected nude mice, as described (see 2.5), were used as the coating antigen. A total of 5,000 trophozoites in 0.05 mL of PBS were added to alternate rows of wells in a 96-(round bottom) well, polyvinyl chloride, microtitre plate (Dynatech-Fisher Scientific, Toronto, ON). The remainder of the wells were left blank. The plates were frozen at −20°C until use.

2.12.2 Assay Procedure

The pre-coated plates were allowed to thaw overnight at 4°C. Unbound sites in all wells were blocked by incubation with NGS-PBS for 1 hour at room temperature. The wells were then washed 5 times with PBS-Tw. Serum samples were diluted 1:50 with NGS-PBS and 0.05 mL aliquots of the dilute samples were added to duplicate antigen-coated wells and duplicate blank wells. After a 3 hour incubation at room temperature, the wells were emptied and washed 5 times with PBS-Tw. Biotinylated-goat anti-mouse IgG (Amersham Canada, Ltd., Oakville, ON) was diluted 1:500 with NGS-PBS, and 0.05 mL of the dilute conjugate was incubated in each well for 3 hours at room temperature. The wells were then emptied and washed 5 times with PBS-Tw. A dilution containing 50,000 counts per minute (cpm) of ^{125}I-Streptavidin (specific activity - 20 μCi per μg - Amersham Canada, Ltd., Oakville, ON) in 0.05 mL of NGS-PBS was incubated

in each well for 3 hours at room temperature. The wells were then emptied and washed 5 times with PBS-Tw. The amount of bound radioactivity in each well was determined using an LKB Wallac 1282 CompuGamma Universal Gamma Counter. The average cpm in the duplicate blank wells was subtracted from the average cpm in the duplicate antigen-coated wells to obtain the "Δ-cpm" result.

2.12.3 Specificity

The isotype specificity of the biotinylated-goat anti-mouse IgG antibody was tested by a direct IRMA using the purified immunoglobulin proteins described (see 2.10.3). The reagent was found to cross-react with IgMκ, IgAκ and isolated κ chains. Therefore, the reagent was adsorbed by affinity chromatography on a column consisting of a mouse monoclonal IgMκ coupled to Sepharose 4B. This procedure succeeded in removing the cross-reacting contaminants, as determined by repeating the specificity IRMA using the adsorbed reagent. Conditions for the IRMA for IgG anti-*G.muris* antibody were optimized using the adsorbed biotinylated-goat anti-mouse IgG antibody. The optimal dilution of this reagent was found to be 1:500.

2.12.4 Standard Curve and Determination of Antibody Activity

A standard curve of antibody activity was constructed using a CBA/J hyperimmune anti-*G. muris* antiserum, prepared as described (see 2.8). This antiserum was assigned a value of 10,000 arbitrary antibody units per mL. A standard curve was constructed for each group of sera assayed. A sigmoidal curve was fit to the standard curve as described

(see 2.10.4). An example from one experiment is provided (Figure 1). The amount of antibody activity, in arbitrary antibody units per mL, in a serum sample was computed from its Δcpm result by interpolation of the fitted standard curve.

2.13 Analysis of the Lipid Components of G. muris

2.13.1 Extraction of Lipids from G. muris

Trophozoites were isolated from the intestines of infected nude mice, as described (see 2.5). Lipid components of the trophozoites were extracted using a technique previously described by Magnanni et al. (1981). Trophozoites (60 - 90 X 10^6) were disrupted by resuspension in 3 mL of water, followed by three 10-second bursts of sonication from a Braun Sonic 2000 probe sonicator. The disrupted trophozoites were added to 10.8 mL of methanol (Fisher Scientific, Toronto, ON), following which 5.4 mL of chloroform (Caledon Laboratories, Georgetown, ON) was added. The mixture was stirred for 30 minutes at room temperature and then centrifuged at 12,000 x g for 10 minutes. The supernatant fluid was saved. The pelleted material was resuspended in 2 mL of water and re-extracted with a mixture of 6 mL of methanol and 3 mL of chloroform. After stirring for 30 minutes at room temperature, the mixture was centrifuged, as before, and the supernatant fluid was added to that from the first extraction. The solvent in the combined extracts was evaporated using a Buchi Rotating Evaporator. The residue was re-dissolved in 2 mL of chloroform:methanol (2:1, volume:volume). The solvent was evaporated again, under a stream of dry nitrogen, and the final residue was

redissolved in a minimum volume of chloroform:methanol (2:1) for application to nitrocellulose or thin layer chromatography plates.

2.13.2 Dot Blot Assay

A qualitative, solid-phase, "dot-blot" assay (Costello and Green 1986) was used to detect antibody binding to lipid components of G. muris. The chloroform:methanol extract of trophozoites was applied to squares of nitrocellulose (Schleicher and Schuell - Keene, NH) using a Hamilton syringe. Approximately 2 μL of extract was applied to a spot, the solvent was allowed to evaporate, and the process was repeated until a total volume of 20 μL, containing 5 X 10^6 trophozoite equivalents, was applied. The nitrocellulose squares were allowed to dry thoroughly. Unbound sites were blocked by incubation in NGS-PBS. The squares were incubated with dilutions of sera, in heat-sealed plastic sacs (Scotchbrand Pouch Sealer, Fisher Scientific, Toronto, ON) for 3 hours at room temperature.. Constant agitation was provided by taping the sacs to an automatic rocking platform (Ames Aliquot Mixer, Miles Scientific, Naperville, IL). The squares were washed with PBS-Tw, following which they were incubated with goat anti-mouse IgM - horse radish perodixase (KPL - Mandel Scientific Co., Rockwood, ON), diluted 1:500 with NGS-PBS (1 mL per square). The squares were again washed with PBS-Tw and then placed in a substrate solution which was prepared as follows: 60 mg of HRP Color Development Reagent containing 4-chloro-1-naphthol (Bio-Rad Laboratories, Richmond, CA) was dissolved in 20 mL of ice cold methanol; 60 μL of 30% hydrogen peroxide (Caledon Laboratories, Georgetown, ON) was added to 100 mL of PBS; the two solutions were mixed together and used immediately. Colour development

was allowed to proceed for 15 minutes at room temperature. The reaction was terminated by removing the squares from the substrate solution and rinsing them with distilled water. The squares were allowed to dry thoroughly, during which time the background colour faded away.

2.13.4 Thin Layer Chromatography and Immunoblotting

For staining, thin layer chromatography (TLC) was performed using glass-backed, silica gel GF TLC plates (Analtech Inc., Newark, DE). A sample of 0.05 mL of the chloroform:methanol extract, containing 9 X 10^6 trophozoite equivalents, was spotted 1 cm from the bottom of the plate. Plates were developed in a TLC chamber containing 200 mL of the following solvent system: chloroform:methanol:0.25% KCl (60:35:8) (Magnani et al. 1981). For detection of lipids, the thin layer chromatogram was incubated in a tank of iodine vapour (Christie 1982). For detection of glycolipids, an orcinol-ferric chloride-sulphuric acid reagent (Vioque 1984) was used: 0.1 x g of ferric chloride was dissolved in 10 mL of 10% (volume:volume) H_2SO_4; 0.6 x g of orcinol (Sigma Chemicals, St. Louis, MO) was dissolved in 10 mL of absolute ethanol (Consolidated Alcohols, Ltd., Toronto, ON); 10 mL of the ferric chloride solution was mixed with 1 mL of the orcinol solution and this reagent was sprayed, in the form of a fine mist, onto the chromatogram, which was then heated at 100°C for 15 minutes.

For immunoblotting (Brockhaus et al. 1981), thin layer chromatography was performed using aluminum-backed, silica gel 60 TLC plates (E. Merck - BDH Chemicals, Toronto, ON). A sample of 0.05 mL of the chloroform:methanol extract, containing 30 X 10^6 trophozoite equivalents, was spotted, and the plates were developed as described

above. After developing, the chromatogram was dried, and then immersed in 30 mL of 0.1% polyisobutylmethacrylate (Polysciences, Inc., Warrington, PA) in hexanes (BDH Chemicals, Toronto, ON). The chromatogram was dried for 2 minutes and unbound sites on the silica gel were blocked by incubation with 30 mL of NGS-PBS for 1 hour at room temperature. The chromatogram was then incubated with 30 mL of a 1:25 dilution of serum, for 3 hours at room temperature, with occasional, gentle agitation. The chromatogram was washed with PBS and then incubated with 30 mL of goat anti-mouse IgM - horse radish peroxidase (KPL - Mandel Scientific Co., Rockwood, ON), diluted 1:500 with NGS-PBS. The chromatogram was then washed with PBS and incubated with 50 mL of the substrate solution described above (see 2.13.2). Colour development was allowed to proceed overnight at room temperature, following which the chromatogram was rinsed briefly with water and allowed to dry.

2.14 SDS-PAGE and Western Blotting

2.14.1 Preparation of Antigens

Trophozoites were isolated from the intestines of infected nude mice, as described (see 2.5), and were resuspended in PBS at a concentration of 2 X 10^8 trophozoites per mL. The suspension was mixed with an equal volume of 2X sample buffer (20% glycerol (volume/volume), 4% sodium dodecyl sulphate (weight/volume), 0.1% 2-mercaptoethanol (volume/volume), 0.0025% bromphenol blue (weight/volume) in 0.125 M Tris-HCl buffer, pH 6.8), and placed in a boiling water bath for 2 minutes.

The sample was aliquoted and frozen at -20°C until use, at which time it was thawed for 2 minutes at 40°C.

Cysts were isolated from the feces of infected nude mice, as described (see 2.3), and were resuspended in saline at a concentration of 4×10^7 cysts per mL. The suspension was mixed with an equal volume of 2X sample buffer, placed in a boiling water bath for 5 minutes, aliquoted and frozen at -20°C until use. The sample was thawed for 2 minutes at 40°C immediately prior to use.

2.14.2 SDS-PAGE

Sodium dodecyl sulphate-polyacrylamide gel electrophoresis was performed according to the method of Laemmli (1970), using a 10% separating gel and a 4% stacking gel. All reagents and equipment were purchased from Bio-Rad Laboratories (Richmond, CA).

Samples were loaded into one well, which was 13.2 cm wide; 100×10^6 trophozoite equivalents in 1 mL of sample buffer, or 20×10^6 cyst equivalents in 1 mL of sample buffer, were loaded. In addition, 0.02 mL of prestained molecular weight standards (Bio Rad Laboratories, Richmond, CA) were loaded into one 0.8 cm-wide well on the same gel. A constant voltage of 100V was applied to the gel until the samples had completely entered the stack, at which time the voltage was lowered to 50V. Electrophoresis was continued (overnight) until the bromphenol blue dye front had migrated at least 12 cm from the origin of the separating gel.

2.14.3 Western Blotting

Protein transfer from SDS-gels to nitrocellulose sheets (Schleicher and Schuell, Keene, NH) was performed according to the method

of Towbin et al. (1979), using a Trans Blot Cell (Bio Rad Laboratories, Richmond, CA) and the following buffer: 0.02 M Tris, 0.15 M glycine in 20% methanol (volume/volume). A constant current of 150 mAmps was applied to the system for 24 hours at 4°C. The success of the transfer was verified by the presence of the prestained molecular weight standards on the nitrocellulose sheet.

The portion of the nitrocellulose sheet to which C. muris antigens had been transferred was cut into strips, 0.8 cm wide. The strips were incubated with dilutions of sera in heat-sealed plastic sacs (Scotchbrand Pouch Sealer, Fisher Scientific, Toronto, ON) for 3 hours at room temperature. Constant agitation was provided by taping the sacs to an automatic rocking platform (Ames Aliquot Mixer, Miles Scientific, Naperville, IL). The strips were washed with PBS-Tw, following which they were incubated with goat anti-mouse IgG-alkaline phosphatase, goat anti-mouse IgA-alkaline phosphatase or goat anti-mouse IgM alkaline phosphatase (5 mL of 1:500 conjugate in NGS-PBS, per strip, - (KPL) - Mandel Scientific Co., Rockwood, ON) overnight at room temperature. The strips were washed with PBS-Tw and colour development was accomplished using the following substrate solution: 30 mg of p-nitroblue tetrazolium chloride (Sigma Chemicals, St. Louis, MO) was dissolved in 1 mL of 70% dimethyl-formamide (DMF - Sigma Chemicals, St. Louis, MO); 15 mg of 5-bromo-4-chloro-3-indolyl phosphate (Sigma Chemicals, St. Louis, MO) was dissolved in 1 mL of 100% DMF; these two solutions were added to 100 mL of 0.1M sodium bicarbonate buffer, pH 9.8, containing 0.01 M magnesium chloride; the substrate solution was used immediately, and colour development was allowed to proceed, in the dark, for 15 minutes. The reaction was

terminated by removing the strips from the substrate solution, and rinsing them with distilled water.

2.15 Statistics

5 mice were used for each experimental group. Mean values for cyst counts, immunoglobulin levels and antibody responses are given as the geometric mean values. The range of the standard error of the mean (Range of SEM) was calculated by taking the antilogarithms (base 10) of the values resulting from the addition, and subtraction, of the logarithm of the SEM to, and from, the logarithm of the geometric mean.

In some experiments, the statistical significance of the difference between two mean values was tested. First an F-test was performed to determine the similarity of the variances of the two groups. If the variances were similar, then Student's t-test was performed. If the variances were different, then the non-parametric Mann-Witney U test was performed. A standard text book (Mendenhall 1979) was used as a reference for statistical procedures.

Chapter 3

RESULTS AND DISCUSSION

3.1 Adoptive Transfer of Cells to CBA/N Mice to Study the Cellular Requirements for Elimination of G. muris

The purpose of these experiments was to study the cellular basis for susceptibility to chronic giardiasis in CBA/N mice. The hypothesis was that B cells and antibody are crucial for elimination of this parasite, and that it is the B cell defect of CBA/N mice that renders them unable to eliminate G. muris. The methods used to test this hypothesis involved the adoptive transfer of cells from immunocompetent, histocompatible, CBA/Ca mice, that eliminate G. muris, to non-irradiated and irradiated CBA/N mice. The objective was to reconstitute the ability to eliminate G. muris and attempt to identify the cell type that was responsible for this reconstitution.

It has been shown that non-irradiated CBA/N mice can be reconstituted with lymphoid cells from CBA/Ca mice (Scher et al. 1975b; Volf et al. 1978). The reconstituted CBA/N mice were responsive to type II T-independent antigens (Scher et al. 1975b; Quintans, McKearn and Kaplan 1979), and contained clonable B cells (Paige et al. 1979) and B

cells capable of responding to LPS (Volf et al. 1978) and anti-IgM antibody (Udkayakumar, Goud and Subbarao 1988). B cells, which were shown to be of donor origin, persisted in the reconstituted CBA/N mice for many months (Volf et al. 1978; Kincaide et al. 1982; Udkayakumar, Goud and Subbarao 1988).

Engraftment of lymphoid cells in non-irradiated CBA/N mice was restricted to B cells; donor T cells did not persist in these mice (Volf et al. 1978). The successful engraftment of B cells, but not T cells, in non-irradiated CBA/N mice was thought to be due to the availability of space in this lymphoid compartment, resulting from the absence of a mature B cell subset in CBA/N mice (Volf et al. 1978). Fetal liver cells, which contain only immature cells were less efficient than spleen cells at reconstituting CBA/N mice (Quintans, McKearn and Kaplan 1979). Successful engraftment of fetal liver B cells required prior, sublethal irradiation of CBA/N recipients and large numbers of donor cells (Quan et al. 1981). This transplantation barrier to fetal liver cell engraftment was attributed to the presence of defective, B cell progenitors in CBA/N mice; irradiation damage to these cells was thought to allow subsequent engraftment of normal, B cell progenitors present in the donor fetal liver cells (Quintans, McKearn and Kaplan 1979).

Lethally irradiated CBA/N or normal mice, reconstituted with spleen cells from normal mice, were responsive to type II T-independent antigens (Scher et al. 1975b). However, lethally irradiated CBA/N or normal mice, reconstituted with spleen cells from CBA/N mice, were not responsive to these antigens (Scher et al. 1975b). Thus, lethal irradiation, followed by reconstitution with lymphoid cells from normal mice,

corrected the immune defect of CBA/N mice. However, in lethally irradiated recipients, engraftment is not restricted to B cells (Paige et al. 1979).

3.1.1 Results

3.1.1.1 Reconstitution of Non-irradiated CBA/N Mice with Spleen Cells from CBA/Ca Mice

Non-irradiated CBA/N mice were reconstituted with spleen cells from CBA/Ca mice. Each mouse received 50 X 10^6 donor cells, since it was previously shown that this was the optimal dose for reconstitution of responsiveness to the type II T-independent antigen, TNP-Ficoll (Scher et al. 1975b). Three weeks after the injection of cells, the reconstituted CBA/N mice were infected with G. muris cysts. This time interval between reconstitution and infection was allowed, since it was previously shown that three weeks were required for donor spleen cells to settle in the Peyer's patches of recipient CBA/N mice (Volf et al. 1978). The course of G. muris infection in the reconstituted CBA/N mice, and those in intact CBA/N and CBA/Ca mice, is shown in Figure 2A. Both the reconstituted and non-reconstituted CBA/N mice developed chronic giardiasis, while the CBA/Ca mice eliminated the parasite. These results were reproduced in two other separate experiments.

The viability of the donor cells and their ability to reconstitute CBA/N mice as previously described (Scher et al. 1975b) was tested by measuring total serum IgM levels and responsiveness to TNP-Ficoll in the reconstituted mice. Twelve weeks following G. muris infection, the

reconstituted mice and the control mice were treated with metronidazole. This treatment terminated the infection in all of the mice. The mice were then bled, and immunized, intraperitoneally, with 100 μg of TNP-Ficoll in 0.2 mL of PBS (Eldridge et al. 1984). Fifteen days later, the mice were bled. Total serum IgM levels and anti-TNP antibody levels were determined by ELISA as described in the Methods and Materials section (see 2.10-2.11). These data are shown in Table 2. The mean total serum IgM level in the reconstituted CBA/N mice was significantly greater than that in the non-reconstituted CBA/N mice, but was still significantly smaller than that in the CBA/Ca mice. However, the mean level of the antibody response to TNP-Ficoll made by the reconstituted CBA/N mice was quantitatively similar to that made by the CBA/Ca mice. The non-reconstituted CBA/N mice did not make any antibody response to TNP-Ficoll. Thus, the reconstitution of CBA/N mice with spleen cells from CBA/Ca mice was successful within certain parameters. However, this reconstitution did not reconstitute the ability to eliminate G. muris.

3.1.1.2 Reconstitution of Non-irradiated CBA/N Mice with Peyer's Patch and Mesenteric Lymph Node Cells from CBA/Ca Mice.

Since G. muris is an intestinal parasite, and since injected spleen cells localize preferentially in the spleen (Sprent et al. 1985), the above experiment was repeated using cells derived from the gut-associated lymphoid tissue, which are known to preferentially localize at mucosal sites (McDermott and Bienenstock 1979). Peyer's patch and mesenteric lymph node cells were isolated from CBA/Ca mice. Non -

Figure 2

CBA/N mice were reconstituted with (A) $50X10^6$ CBA/Ca splenocytes, or (B) $35X10^6$ CBA/Ca Peyer's patch and mesenteric lymph node cells. Three weeks later (at week 0), the reconstituted mice (●), together with intact CBA/N controls (○), and intact CBA/Ca controls (□), were infected by peroral administration of 5000 G. muris cysts. The course of infection was followed by measuring G. muris cyst output in feces. Each point represents the mean of values from 5 mice. Error bars represent the standard error of the mean. The arrow marks the limit of detection (600 cysts per 8 fecal pellets).

ADOPTIVE TRANSFER OF SPLEEN CELLS (A) AND PEYER'S PATCH AND MESENTERIC LYMPH NODE CELLS (B) TO NON-IRRADIATED CBA/N MICE: COURSE OF G. MURIS INFECTION IN RECONSTITUTED MICE

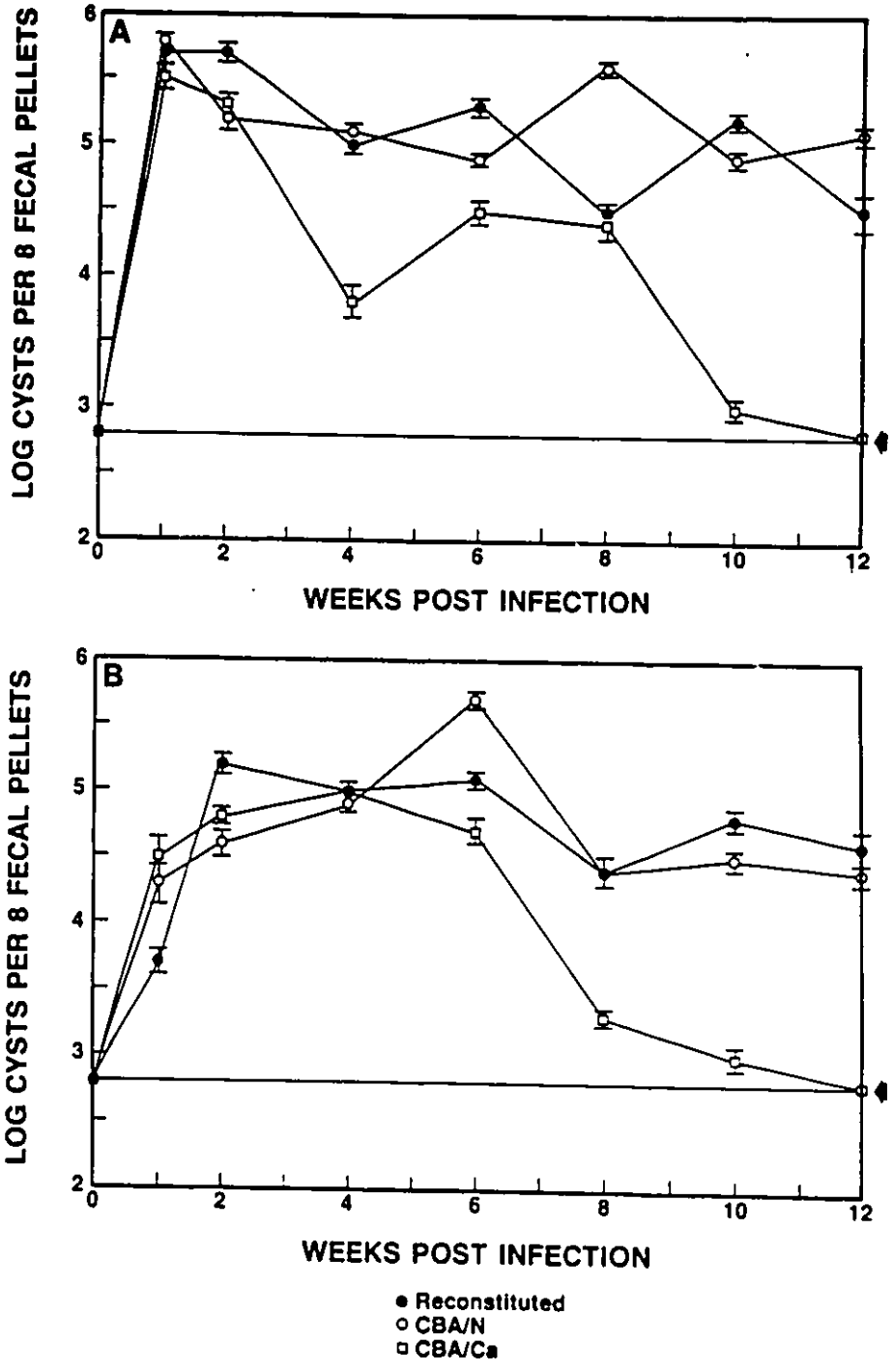


TABLE 2

RECONSTITUTION OF CBA/N MICE WITH SPLEEN CELLS (SPL) AND PEYER'S PATCH AND MESENTERIC LYMPH NODE CELLS (PP/MLN) FROM CBA/Ca MICE

EXPERIMENT MICE	TOTAL SERUM IgM (μg/mL)[c]	ANTI-TNP ANTIBODY (arbitrary units/mL)[c] Preimmune	15 days PI
SPL transfer			
Reconstituted CBA/N[a]	308[e](301-316)[d]	836(810-864)[d]	6038[f](5287-6896)[d]
Non-reconstituted CBA/N[b]	170[g](160-182)	388(348-432)	510[g,h](446-583)
Non-reconstituted CBA/Ca[b]	408 (401-414)	661(610-716)	6412 (6158-6676)
PP/MLN transfer			
Reconstituted CBA/N[a]	238[e](232-244)	N/D	8344[f] (7659-9090)
Non-reconstituted CBA/N[b]	128[g](121-135)	N/D	702[g] (636-775)
Non-reconstituted CBA/Ca[b]	341 (331-350)	N/D	13490(11849-15358)

a CBA/N mice were reconstituted with $50X10^6$ spleen cells (SPL transfer) or $35X10^6$ Peyer's patch and mesenteric lymph node cells (PP/MLN transfer). Three weeks later, the mice were infected with C. muris. Twelve weeks after infection, the mice were bled to obtain pre-immune sera, then were immunized with 100 μg of TNP-Ficoll. Fifteen days later, the mice were bled; total serum IgM levels and anti-TNP antibody levels were measured using these sera.

b Non-reconstituted CBA/N and CBA/Ca mice were treated as described above, except they did not receive donor cells.

c Geometric mean of 5 mice.

d Range of SEM (anti-logs of values resulting from subtraction, and addition, of the log of SEM from, and to, the log of the geometric mean).

e Significantly different from both CBA/N and CBA/Ca $p<0.01$.

f Not significantly different from CBA/Ca $p>0.1$.

g Significantly different from CBA/Ca $p<0.01$.

h Not significantly different from the corresponding pre-immune value $p>0.1$.

N/D Not done.

PI Post immunization with TNP-Ficoll.

irradiated CBA/N mice received 35 X 10^6 of these cells, each. The lower number of donor cells per recipient mouse in this experiment was due to the lower yield of cells from Peyer's patches and mesenteric lymph nodes, relative to spleen. The reconstituted CBA/N mice, and non-reconstituted CBA/N and CBA/Ca mice, were infected with G. muris three weeks later. The course of infections in these mice is shown in Figure 2B. The CBA/N mice reconstituted with gut-derived lymphoid cells failed to eliminate G. muris. The non-reconstituted CBA/N mice also developed chronic giardiasis, while the CBA/Ca mice eliminated the parasite.

Total serum IgM levels and responses to immunization with TNP-Ficoll were determined, as described above. These data are shown in Table 2. The results of the Peyer's patch/mesenteric lymph node cell transfer were essentially the same as those of the spleen cell transfer. Total serum IgM was partially reconstituted, and responsiveness to TNP-Ficoll was fully restored. Although the mean level of the anti-TNP antibody response was greater in CBA/Ca mice compared to the reconstituted CBA/N mice, this difference was not statistically significant. Thus, reconstitution of CBA/N mice with Peyer's patch and mesenteric lymph node cells from CBA/Ca mice, failed to reconstitute the ability to eliminate G. muris.

3.1.1.3 Reconstitution of Irradiated CBA/N and CBA/Ca Mice with Spleen Cells from CBA/N and CBA/Ca Mice.

I next considered the hypothesis that the defect of CBA/N mice that renders them susceptible to chronic giardiasis is in a non-mature B

cell or non-B cell population. Thus, reconstitution of the ability to eliminate G. muris may have been blocked by the presence of defective B cell progenitors or other defective cells in CBA/N mice (Quintans, McKearn and Kaplan 1979). In the following experiments, mice were lethally irradiated immediately prior to reconstitution. Both CBA/N and CBA/Ca mice served as lethally irradiated recipients. Donor spleen cells were derived from both CBA/N and CBA/Ca mice; 50 X 10^6 donor cells were injected into each recipient. Mice were infected with G. muris three weeks after reconstitution. Intact CBA/N and CBA/Ca mice served as controls. The course of infections in these mice is shown in Figure 3. Lethally irradiated mice, reconstituted with CBA/Ca spleen cells, eliminated G. muris infection. In contrast, lethally irradiated mice, reconstituted with CBA/N spleen cells, developed chronic giardiasis. These results were consistent whether CBA/N mice or CBA/Ca mice were the lethally irradiated recipients, and were reproduced in two other separate experiments.

Total serum IgM levels and responses to immunization with TNP-Ficoll were determined, as described above. These data are shown in Table 3. Lethally irradiated CBA/N mice, reconstituted with CBA/N spleen cells, retained the CBA/N phenotype. Similarly, lethally irradiated CBA/Ca mice, reconstituted with CBA/Ca spleen cells, retained the CBA/Ca phenotype. Lethally irradiated CBA/N mice, reconstituted with CBA/Ca spleen cells, developed the CBA/Ca phenotype: their mean total serum IgM level was equivalent to that of intact CBA/Ca mice, and their mean anti-TNP antibody response was quantitatively similar to that of intact CBA/Ca mice. Lethally irradiated CBA/Ca mice, reconstituted with CBA/N spleen cells, had an intermediate phenotype: their mean serum IgM level was

significantly less than that of intact CBA/Ca mice, but significantly greater than that of intact CBA/N mice, and there was a small, but significant, antibody response to immunization with TNP-Ficoll. Thus, reconstitution of lethally irradiated mice with spleen cells from CBA/Ca mice reconstituted the ability to eliminate G. muris. However, reconstitution of lethally irradiated mice with spleen cells from CBA/N mice failed to reconstitute this ability.

3.1.1.4 Reconstitution of Irradiated CBA/N Mice with Mixtures of Splenic B cells and T cells from CBA/N and CBA/Ca Mice

I next attempted to identify the cell type in CBA/Ca spleen that was responsible for transferring, to lethally irradiated mice, the ability to eliminate G. muris. I hypothesized that this would be a lymphocyte, either a B cell or a T cell. This hypothesis was tested by reconstituting lethally irradiated CBA/N mice with mixtures of cell subsets from CBA/N and CBA/Ca mice. CBA/Ca spleen cells were selectively depleted of T cells by treatment with anti-Thy1.2 antibody and complement; CBA/N spleen cells were selectively depleted of B cells by panning on anti-immunoglobulin antibody-coated petri dishes. Lethally irradiated CBA/N mice were reconstituted with a total of 50 X 10^6 cells, each: 25 X 10^6 T cell-depleted, CBA/Ca spleen cells plus 25 X 10^6 B cell-depleted, CBA/N spleen cells. The reciprocal experiment was also conducted. That is, lethally irradiated CBA/N mice were reconstituted with 25 X 10^6 B cell-depleted, CBA/Ca spleen cells plus 25 X 10^6 T cell-depleted, CBA/N spleen cells. The reconstituted mice, together with intact CBA/N and CBA/Ca control mice,

Figure 3

(A) CBA/N mice and (B) CBA/Ca mice were lethally irradiated by exposure to 1000 rads from a ^{137}Cs source. Immediately following the irradiation, the mice were reconstituted with $50X10^6$ CBA/Ca splenocytes (O), or $50X10^6$ CBA/N splenocytes (●). Three weeks later (at week 0), the mice were infected by peroral administration of 5000 *G. muris* cysts. The course of infection was followed by measuring *G. muris* cyst output in feces. Each point represents the mean of values from 5 mice. Error bars represent the standard error of the mean. The arrow marks the limit of detection (600 cysts per 8 fecal pellets).

ADOPTIVE TRANSFER OF SPLEEN CELLS TO IRRADIATED CBA/N (A) AND CBA/Ca (B) MICE: COURSE OF G. MURIS INFECTION IN RECONSTITUTED MICE

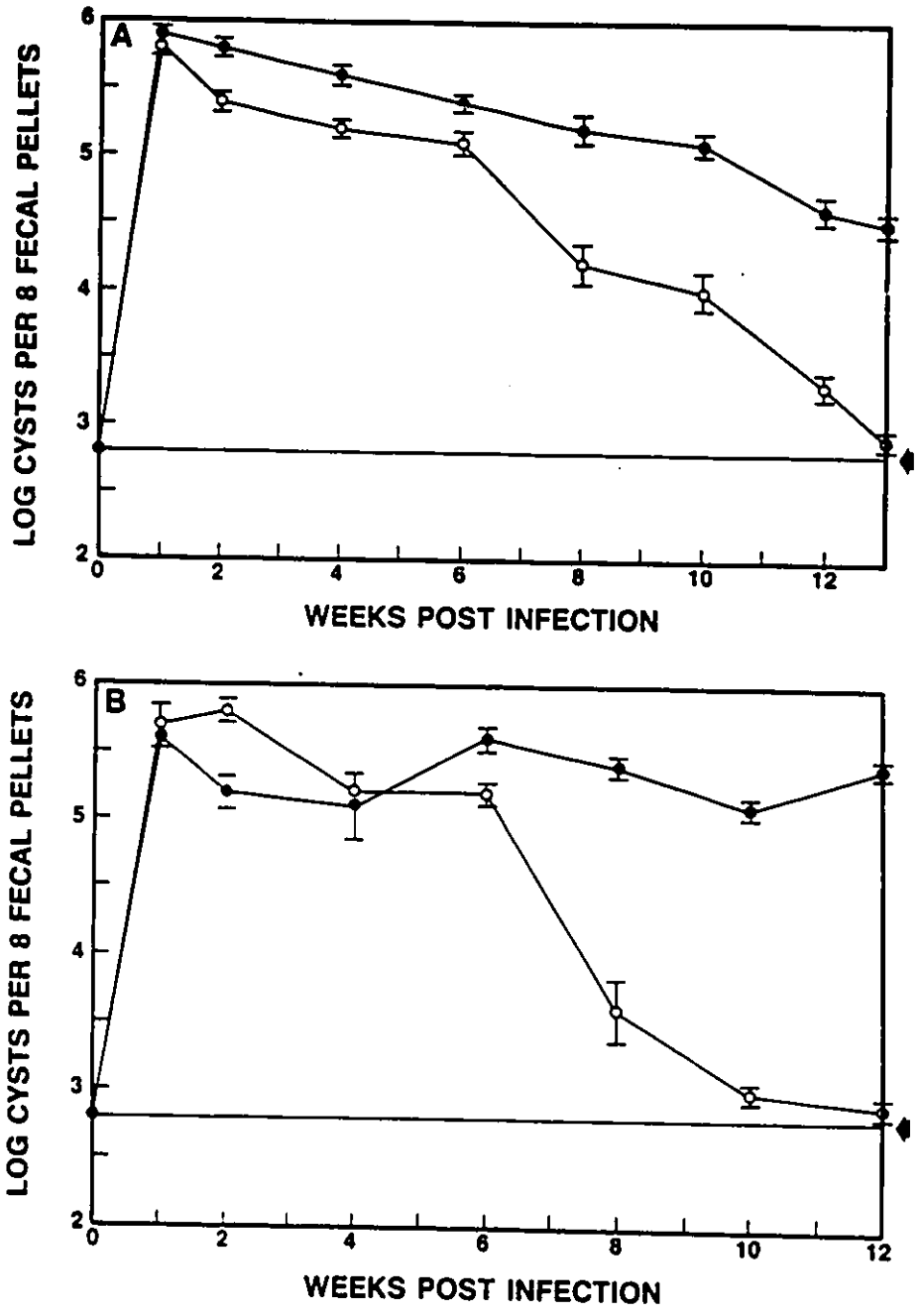


TABLE 3

RECONSTITUTION OF IRRADIATED MICE WITH CBA/N AND CBA/Ca SPLEEN CELLS

STRAIN OF DONOR CELLS	STRAIN OF MICE	TOTAL SERUM IgM (μg/mL)[c]	ANTI-TNP ANTIBODY (arbitrary units/mL)[c] Pre-immune	15 days PI
CBA/N	CBA/N[a]	72[e,h] (68-76)[d]	N/D	579[e,h] (556-604)[d]
CBA/Ca	CBA/N[a]	275[f] (262-289)	N/D	12,034[f] (11,543-12,546)
—	CBA/N[b]	76 (74-78)	N/D	745 (707-785)
—	CBA/Ca[b]	276 (268-274)	N/D	12,918 (12,592-13,252)
CBA/N	CBA/Ca[a]	374[g,h] (347-404)	523 (495-552)	1,184[g,h,i] (930-1,508)
CBA/Ca	CBA/Ca[a]	703[f] (680-726)	826 (773-884)	6,848[f,i] (6,547-7,163)
—	CBA/N[b]	102 (94-111)	190 (175-206)	165[j] (151-181)
—	CBA/Ca[b]	748 (716-782)	762 (716-812)	5,080[i] (4,969-5,194)

a Mice were lethally irradiated, then reconstituted with $50X10^6$ spleen cells from the designated strain. Three weeks later, the mice were infected with C. muris. Twelve weeks after infection, the mice were bled to obtain pre-immune sera, then were immunized with 100 μg of TNP-Ficoll. Fifteen days later, the mice were bled; total serum IgM levels and anti-TNP antibody levels were measured using these sera.

b Intact CBA/N and CBA/Ca mice were treated as described above, except they were neither irradiated, nor reconstituted.

c Geometric mean of 5 mice.

d Range of SEM.

e Not significantly different from CBA/N, $p>0.1$.

f Not significantly different from CBA/Ca, $p>0.1$.

g Significantly different from CBA/N, $p<0.05$.

h Significantly different from lethally irradiated mice reconstituted with CBA/Ca donor cells, $p<0.01$.

i Significantly different from corresponding pre-immune value, $p<0.01$.

j Not significantly different from corresponding pre-immune value, $p>0.1$.

N/D Not done.

PI Post-immunization with TNP-Ficoll.

were infected with G. muris three weeks after the reconstitution. The course of infection in these mice is shown in Figure 4A. None of the reconstituted mice eliminated G. muris. Similar to the intact CBA/N mice, the reconstituted mice developed chronic giardiasis. The intact CBA/Ca mice eliminated the parasite. These results were reproduced in one other separate experiment.

As a control experiment, lethally irradiated CBA/N mice were reconstituted with 25 X 10^6 T cell-depleted, CBA/Ca spleen cells, plus 25 X 10^6 B cell-depleted, CBA/Ca spleen cells. Three weeks later, the reconstituted mice were infected with G. muris. The course of infection in these mice is shown in Figure 4B. Surprisingly, the reconstituted mice failed to eliminate G. muris. These results were reproduced in one other separate experiment. Thus, lethally irradiated CBA/N mice, reconstituted with unmanipulated, CBA/Ca spleen cells, eliminated G. muris (Figure 3). However, lethally irradiated CBA/N mice, reconstituted with T cell-depleted, CBA/Ca spleen cells plus B cell-depleted, CBA/Ca spleen cells, did not (Figure 4B).

Total serum IgM levels and responses to TNP-Ficoll immunization in the lethally irradiated recipients of mixtures of cell subsets were determined, as described above. These data are shown in Table 4. The mean serum IgM level in each group of lethally irradiated, reconstituted mice was comparable to that of intact CBA/Ca mice. The mean anti-TNP antibody response of the lethally irradiated recipients of T cell-depleted, CBA/Ca spleen cells plus B cell-depleted, CBA/N spleen cells, and that of B cell-depleted, CBA/Ca spleen cells plus T cell-depleted, CBA/N spleen cells, were quantitatively similar to that of intact CBA/Ca

Figure 4

CBA/N mice were lethally irradiated by exposure to 1000 rads from a ^{137}Cs source. Immediately following the irradiation, the mice were reconstituted (A) with $25X10^6$ T cell-depleted CBA/Ca splenocytes plus $25X10^6$ B cell-depleted CBA/N splenocytes (■), or with $25X10^6$ T cell-depleted CBA/N splenocytes plus $25X10^6$ B cell-depleted CBA/Ca splenocytes (●), or (B) with $25X10^6$ T cell-depleted CBA/Ca splenocytes plus $25X10^6$ B cell-depleted CBA/Ca splenocytes (▲). Intact CBA/N mice (○) and intact CBA/Ca (□) served as controls. Three weeks later (at week 0), the mice were infected by peroral administration of 5000 G. muris cysts. The course of infection was followed by measuring cyst output in feces. Each point represents the mean of values from 5 mice. The arrow marks the limit of detection (600 cysts per 8 fecal pellets).

ADOPTIVE TRANSFER OF MIXTURES OF T CELL-DEPLETED AND B CELL-DEPLETED SPLENOCYTES FROM C3A/N AND CBA/Ca MICE TO IRRADIATED CBA/N MICE: COURSE OF G. MURIS INFECTION IN RECONSTITUTED MICE

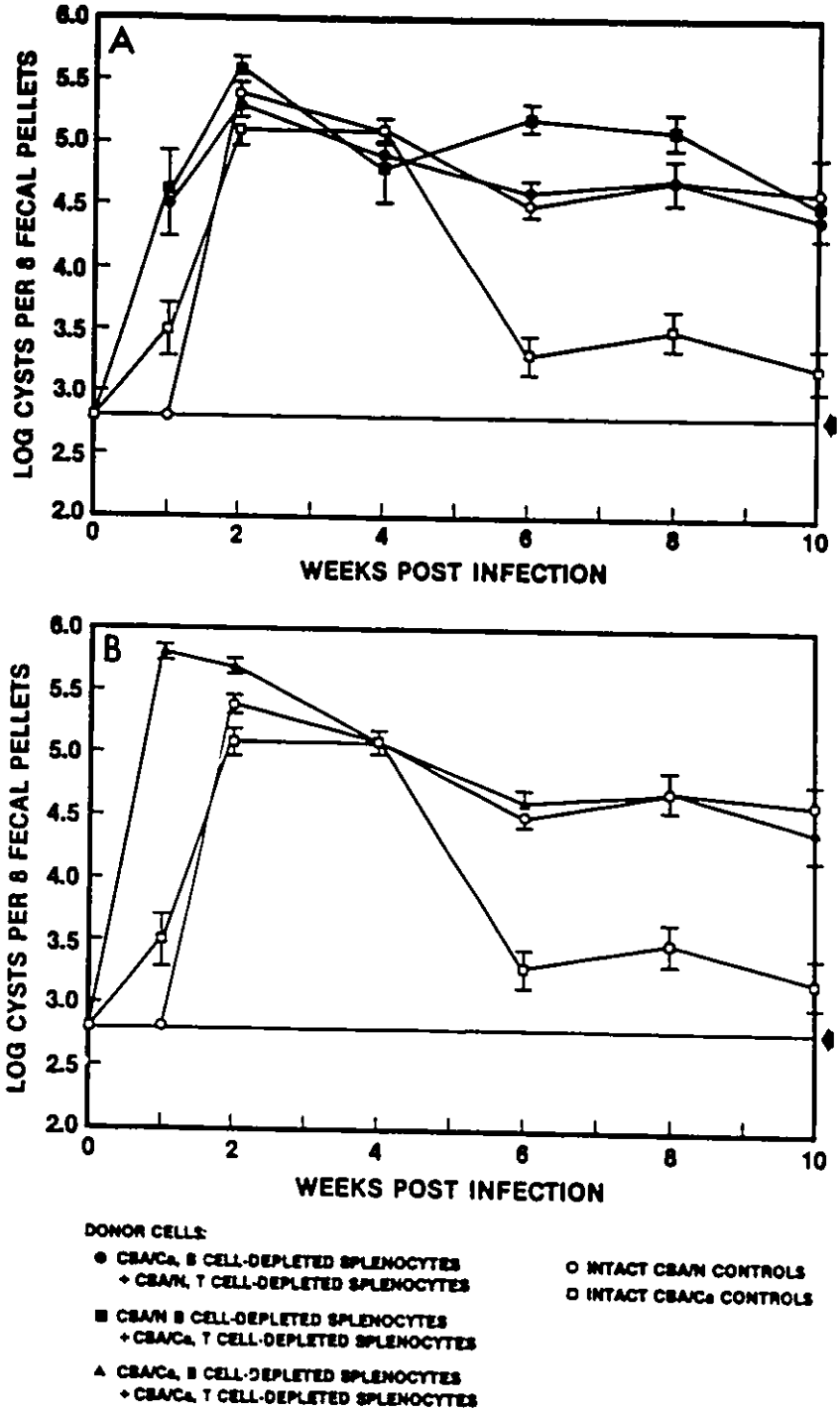


TABLE 4

RECONSTITUTION OF IRRADIATED CBA/N MICE WITH MIXTURES OF T CELL-DEPLETED AND B CELL-DEPLETED SPLEEN CELLS FROM CBA/N AND CBA/Ca MICE

	TOTAL SERUM IgM (μg/mL)[c]	Anti-TNP Antibody (arbitrary units/mL)[c] Pre-immune	15 days PI
Donor cells[a]			
CBA/Ca, T-depleted cells + CBA/N, B-depleted cells	578[e](530-629)[d]	1118(1064-1175)	4034[e,h](3884-4190)[d]
CBA/N, T-depleted cells + CBA/Ca, B-depleted cells	583[e](510-664)	1041 (971-1116)	4048[e,h](3886-4218)
CBA/Ca, T-depleted cells + CBA/Ca, B-depleted cells	643[e](578-717)	960 (938-981)	3707[f,h](3653-3762)
Intact CBA/N controls[b]	89[g] (87-91)	651 (589-720)	491[g,h](4016-4106)
Intact CBA/Ca controls[b]	626 (595-658)	644 (584-710)	4061[h] (4016-4106)

a CBA/N mice were lethally irradiated, then reconstituted with $25X10^6$ T cell-depleted splenocytes plus $25X10^6$ B cell-depleted splenocytes. Three weeks later, the mice were infected C. muris. Twelve weeks after infection, the mice were bled to obtain pre-immune sera, then were immunized with 100 μg of TNP-Ficoll. Fifteen days later, the mice were bled; total serum IgM levels and anti-TNP antibody levels were measured using these sera.

b Intact CBA/N and CBA/Ca mice were treated as described above, except they were neither irradiated, nor reconstituted.

c Geometric mean of 5 mice.

d Range of SEM.

e Not significantly different from intact CBA/Ca controls, $p>0.1$.

f Not significantly different from intact CBA/Ca controls, $p>0.5$.

g Significantly different from intact CBA/Ca controls, $p<0.01$.

h Singificantly different from the corresponding pre-immune value, $p<0.01$.

i Not significantly different from the corresponding pre-immune value, $p>0.1$.

PI Post-immunization with TNP-Ficoll.

mice. The mean anti-TNP antibody response of the lethally irradiated recipients of T cell-depleted, CBA/Ca spleen cells plus B cell-depleted, CBA/Ca spleen cells, was slightly smaller than that of intact CBA/Ca mice. However, this difference was not statistically significant.

3.1.2 Discussion

Previous *in vivo* studies of the cellular requirements for the elimination of *G. muris* demonstrated that T cell-dependent, humoral immune mechanisms are involved in this process. This conclusion was based on the observations that mice deficient in helper T cells, and mice deficient in B cells, developed chronic giardiasis (Heyworth, Carlson and Ermak 1987; Snider et al. 1985). To further investigate the B cell requirement for elimination of *G. muris*, the cellular basis for susceptibility to chronic giardiasis in CBA/N mice was studied.

CBA/N mice fail to eliminate *G. muris* (Snider, Skea and Underdown 1988). These mice express an X-linked, immunodeficiency gene that results in defective B cell maturation and consequent impairment of certain humoral immune responses (Scher 1982a). Their T cell functions are normal (Scher et al. 1975b). Thus, it was my hypothesis that the B cell defect of CBA/N mice renders them susceptible to chronic infection with *G. muris*.

The absence of a subset of mature B cells in CBA/N mice permits their reconstitution with lymphoid cells, in the absence of preparative irradiation (Scher et al. 1975a; Quintans, McKearn and Kaplan 1979). Reconstitution is restricted to B cells (Volf et al. 1978). The reconstituted CBA/N mice are responsive to type II T-independent antigens (Scher et al. 1975b).

Non-irradiated CBA/N mice, reconstituted with spleen cells from CBA/Ca mice, failed to eliminate G. muris (Figure 2A). The transferred cells were viable, since the reconstituted mice made antibody responses to the type II T-independent antigen, TNP-Ficoll, and their serum IgM levels were partially restored (Table 2). In an experiment that was not reported, various numbers of CBA/Ca spleen cells (up to 50 X 10^6) were used to reconstitute non-irradiated CBA/N mice. The results of this experiment demonstrated a dose-dependent increase in total serum IgM levels in the recipient CBA/N mice. Thus, it is possible that greater than 50 X 10^6 CBA/Ca spleen cells would fully reconstitute serum IgM in CBA/N mice. Alternatively, non-irradiated CBA/N mice may possess a partial block to reconstitution of IgM. The latter hypothesis is supported by the observation that 50 X 10^6 CBA/Ca spleen cells were sufficient to fully reconstitute total serum IgM levels in lethally irradiated CBA/N mice (Table 3). Nonetheless, the important result is that non-irradiated CBA/N mice, reconstituted with spleen cells from CBA/Ca mice, failed to eliminate G. muris, even though other immune responses were successfully reconstituted.

The failure of CBA/Ca spleen cells to reconstitute the ability of CBA/N mice to eliminate G. muris may have been due to the tendency of injected spleen cells to localize in the spleen (Sprent et al. 1985). Since G. muris is an intestinal parasite, and since cells derived from the gut-associated lymphoid tissue (GALT) tend to localize at mucosal sites (McDermott and Bienenstock 1979), I considered the hypothesis that GALT-cells may be more effective than spleen cells at reconstituting a protective immune response against G. muris. There are two complications

in the consideration of this hypothesis. First, Volf et al. (1978) showed that, when CBA/Ca spleen cells were injected into non-irradiated CBA/N mice, some of the donor cells did settle in the Peyer's patches of the recipient mice. Second, Eldridge et al. (1983, 1984) showed that the Peyer's patches of CBA/N mice contain B cells with a mature phenotype, that are responsive to TNP-Ficoll. Thus, there is an apparent paradox: there are mature B cells in the Peyer's patches of CBA/N mice, but CBA/N mice fail to eliminate G. muris. Nevertheless, I tested the hypothesis that GALT cells from CBA/Ca mice may transfer, to CBA/N mice, the ability to eliminate G. muris. A mixture of Peyer's patch cells and mesenteric lymph node cells from CBA/Ca mice was injected into non-irradiated CBA/N mice, and the reconstituted mice were infected with G. muris . The results of this experiment were essentially identical to those of the spleen cell transfer experiment. The reconstituted mice failed to eliminate G. muris (Figure 2B). They made normal antibody responses to TNP-Ficoll, and their total serum IgM levels were partially restored (Table 2). These results were not surprising in light of the considerations that were just discussed.

I next considered the hypothesis that the defect of CBA/N mice that renders them susceptible to chronic giardiasis is in a non-mature B cell or non-B cell population, reconstitution of which was blocked by the presence of defective cells. Therefore, I used a protocol that involved preparative irradiation of recipient mice, to eliminate host lymphocytes, including the defective cells, prior to reconstitution (Quintans, McKearn and Kaplan 1979). I found that lethally irradiated recipients of CBA/Ca spleen cells eliminated G. muris, while lethally irradiated recipients of

CBA/N spleen cells developed chronic giardiasis (Figure 3). These results were consistent irrespective of the strain of recipients. Therefore, the defect of CBA/N mice that renders them susceptible to chronic giardiasis is in the radiosensitive, immune tissues and not in the radioresistant, constitutive tissues. That is, the susceptibility of CBA/N mice to chronic giardiasis is not due to an altered gut physiology, for example. The host environment of CBA/N mice did support elimination of G. muris in the presence of immunocompetent CBA/Ca spleen cells.

Total serum IgM levels and anti-TNP-Ficoll antibody responses were fully restored in lethally irradiated CBA/N mice, reconstituted with spleen cells from CBA/Ca mice (Table 3). This supports the hypothesis that there is a partial block to reconstitution of serum IgM in non-irradiated CBA/N mice, that was removed by irradiation. In the homologous transfer experiments (CBA/N spleen cells into irradiated CBA/N mice, and CBA/Ca spleen cells into irradiated CBA/Ca mice), the reconstituted mice retained their phenotype, as expected. However, lethally irradiated CBA/Ca mice, reconstituted with spleen cells from CBA/N mice, had an intermediate phenotype. Their total serum IgM levels were in between those of intact CBA/N and CBA/Ca mice, and they made small, but significant, antibody responses to TNP-Ficoll (Table 3). These results are in agreement with the previous observations of Scher et al. (1975b). The results are consistent with the possibility that some host cells survived the irradiation. It was previously shown that host cells of lethally irradiated, reconstituted mice contributed to subsequent immune responses in the presence of donor cells (Pilarski and Cunningham 1975). Another possibility is that CBA/N mice possess a radioresistant block to

B cell expression, and that CBA/N cells are able to partially recover when transferred to lethally irradiated CBA/Ca mice. Either explanation may account for the intermediate phenotype of the lethally irradiated, CBA/N spleen cell-reconstituted, CBA/Ca mice.

The observation that CBA/Ca spleen cells transferred, to lethally irradiated recipients, the ability to eliminate G. muris, while CBA/N spleen cells failed to do so, indicates that CBA/Ca spleen contains functional elements, required for elimination of G. muris, that are absent or defective in CBA/N spleen. Both B cells and T cells are required for elimination of G. muris (Snider et al. 1985; Heyworth, Carlson and Ermak 1987), and other T cell functions in CBA/N mice are normal (Scher et al. 1975a). Therefore, I considered the hypothesis that it was the defective B cells that precluded the ability of CBA/N spleen cells to transfer resistance to G. muris.

To test this hypothesis, I selectively depleted T cells from CBA/Ca spleen cells, to obtain a population enriched for CBA/Ca B cells, and I selectively depleted B cells from CBA/N spleen cells, to obtain a population enriched for CBA/N T cells. The two populations were mixed together and used to reconstitute lethally irradiated CBA/N mice. The reciprocal experiment was also conducted. That is, populations enriched for CBA/N B cells and CBA/Ca T cells were used to reconstitute lethally irradiated CBA/N mice. I expected that mice reconstituted with CBA/Ca B cells and CBA/N T cells would eliminate G. muris, while mice reconstituted with CBA/N B cells and CBA/Ca T cells would fail to do so. These were not the results observed. Surprisingly, both groups of reconstituted mice developed chronic giardiasis (Figure 4A).

There are several possible explanations for these results. First, CBA/N mice may have defects in both B cells and T cells that contribute to their susceptibility to chronic giardiasis. Therefore, both CBA/Ca B cells and CBA/Ca T cells may be required for elimination of the parasite. Second, there may be a lack of cooperation between B cells and T cells of the two different strains. This possibility was not considered to be likely, since CBA/N and CBA/Ca mice are histocompatible, and in other systems, B cells and T cells from the two strains cooperated (Scher, Berning and Asofsky 1979; Boswell et al. 1980). Third, CBA/N donor cells may contain suppressor elements that inhibit the response of the CBA/Ca cells. This possibility was not considered to be likely since, in other systems, there was no suppression, by CBA/N cells, of responses by CBA/Ca cells (Scher, Berning and Asofsky 1979; Ono et al. 1983). Finally, a non-B, non-T cell type, or non-conventional B cell or T cell, may have been depleted by both the B cell depletion and T cell depletion procedures.

The following experiment was designed to test these hypotheses. CBA/Ca spleen cells were divided into two portions; one portion was depleted of T cells, and the other of B cells. The two portions, one enriched for CBA/Ca B cells, the other enriched for CBA/Ca T cells, were mixed together and injected into lethally irradiated CBA/N mice. Surprisingly, the reconstituted mice failed to eliminate G. muris (Figure 4B). Thus, lethally irradiated CBA/N mice, reconstituted with unmanipulated, CBA/Ca spleen cells, eliminated G. muris (Figure 3A), while those reconstituted with B cell-depleted, CBA/Ca spleen cells plus T cell-depleted, CBA/Ca spleen cells, failed to do so (Figure 4B).

These results support the fourth hypothesis, described above. That is, there is a non-conventional cell type that is required for elimination of G. muris, that has the following characteristics: 1) it is absent or defective in CBA/N mice; 2) it is present and functional in CBA/Ca spleen, and 3) it is depleted by both the T cell depletion and B cell depletion techniques used. What might this cell type be, and why might it have been lost by procedures designed to selectively deplete only T cells and only B cells? It may be a very adherent cell, that bound to the polystyrene surfaces of the culture tubes and petri dishes used during the depletion procedures. Alternatively, it may be a cell that is present at a very low frequency in CBA/Ca spleen. Although the depletion procedures were selective for T cells and B cells, they involved large, non-specific losses of cells. The T cell depletion procedure resulted in the depletion of greater than 97% of T cells, but also resulted in the removal of approximately 50% of B cells, and approximately 60% of cells that were not stained by either FITC-anti Thy 1.2 antibody or FITC-anti-Ig antibody (unstained cells). The B cell depletion procedure resulted in the depletion of greater than 95% of B cells, but also resulted in the removal of approximately 45% of T cells and approximately 50% of unstained cells. I attempted to control for these non-specific losses by transferring a constant number of recovered cells, that is, 25 X 10^6 of the T-depleted cells plus 25 X 10^6 of the B-depleted cells. The T cell-depleted populations consisted of greater than 75% B cells, and less than 5% T cells. The B cell-depleted populations consisted of greater than 75% T cells, and less than 5% B cells. Thus, both populations contained approximately 20% unstained cells. In spite of this precaution, if the

crucial cell type in CBA/Ca spleen was present at a very low frequency, then its numbers may have been reduced to negligible quantities due to non-specific losses. If, in addition, this cell was adherent, then the probability of its loss would be further increased.

A possible candidate for the cell type that appears to be crucial for elimination of G. muris is the Lyl+ B cell. There are several reasons for formulating this hypothesis. First, CBA/N mice lack Lyl+ B cells (Hayakawa, Hardy and Herzenberg 1986; Herzenberg et al. 1986). Second, Lyl+ B cells are present at a very low frequency in normal, adult mouse spleen; Lyl+ B cells constitute less than 2% of splenic B cells (Herzenberg et al. 1986). Third, Lyl+ B cells tend to adhere to plastic surfaces (L. Herzenberg, personal communication). Finally, Lyl+ B cells contribute to intestinal B cell populations (Kroese et al. 1989). Hayakawa et al. (1985) found that spleen cells from normal, adult mice failed to reconstitute Lyl+ B cells when transferred to lethally irradiated CBA/N recipients. I found that spleen cells from adult CBA/Ca mice reconstituted the ability of lethally irradiated CBA/N mice to eliminate G. muris (Figure 3A). These results appear to be inconsistent with the hypothesis that Lyl+ B cells are the crucial cells for elimination of G. muris. However, Hayakawa et al. (1985) used only 1 X 10^6 donor spleen cells per recipient mouse, whereas I used 50 X 10^6 cells. It is possible that the larger number of donor spleen cells may have contained sufficient numbers of Lyl+ B cells to reconstitute this B cell subset in lethally irradiated, CBA/N mice.

The results of assays to determine total serum IgM levels and anti-TNP-Ficoll antibody responses in lethally irradiated CBA/N mice,

reconstituted with spleen cell subsets, were somewhat surprising. As expected, the recipients of T cell-depleted, CBA/Ca spleen cells (normal B cells) plus B cell-depleted, CBA/N spleen cells (normal T cells) had normal levels of total serum IgM and made normal antibody responses to TNP-Ficoll (Table 4). However, unexpectedly the recipients of B cell-depleted, CBA/Ca spleen cells (normal T cells) plus T cell-depleted, CBA/N spleen cells (defective B cells) also had normal levels of total serum IgM and made normal antibody responses to TNP-Ficoll One possible explanation for these results is that the B cell depletion procedure was incomplete, and the CBA/Ca B cells that remained in the transferred CBA/Ca T cell inoculum, expanded in the lethally irradiated CBA/N recipients and replaced the CBA/N B cells that were co-transferred This explanation is consistent with the observations of Sprent and Bruce (1984) These investigators reconstituted lethally irradiated mice with mixtures of CBA/N bone marrow and normal (B6) bone marrow They found that virtually all of the B cells present in the reconstituted mice were of B6 origin The normal (B6) B cells expanded and replaced the co-transferred CBA/N B cells

As expected, the recipients of T cell-depleted CBA/Ca spleen cells and B-cell-depleted, CBA/Ca spleen cells had normal levels of total serum IgM (Table 4) Their antibody responses to TNP-Ficoll were slightly smaller than those of intact CBA/Ca mice However, this difference was not statistically significant

Thus, although some immune elements in lethally irradiated CBA/N mice were restored by reconstitution with CBA/N and CBA/Ca cell subsets, the ability to eliminate G. muris was not restored Therefore, there is

something non-conventional about the cellular requirements for elimination of G. muris. The results neither prove nor rule out the hypothesis that the B cell defect of CBA/N mice renders them susceptible to chronic giardiasis. Certainly, B cells are required for elimination of G. muris (Snider et al. 1985), and the conventional B cell defect of CBA/N mice may contribute to their failure to eliminate this parasite. However, there may be an additional defect in CBA/N mice that contributes to their susceptibility to chronic giardiasis. That is, there may be another cell type that is crucial for elimination of G. muris. Alternatively, the defect may be in a non-conventional B cell subset, for example, the Lyl+ B cell subset. In either case, the crucial cell type appears to be present at a very low frequency in CBA/Ca spleen, and may be plastic-adherent. Thus, the ability of CBA/Ca spleen cells to transfer, to lethally irradiated recipients, the ability to eliminate G. muris, was lost upon manipulation of the donor cells

3.1.3 Suggestions for Future Experiments

To test the hypothesis that the cell type crucial for elimination of G. muris is present at a low frequency in CBA/Ca spleen, the dose-response relationship between the number of CBA Ca spleen cells transferred to lethally irradiated CBA/N mice, and the ability of the reconstituted mice to eliminate G. muris, should be examined. In addition, the depletion/transfer experiments should be repeated as homologous transfer experiments. That is, T-depleted CBA Ca spleen cells and B-depleted CBA/Ca spleen cells should be transferred to lethally

irradiated CBA/Ca mice. This experiment would control for the possibility that small numbers of the crucial cell type may be able to expand in the syngeneic environment, but not in the CBA/N environment. This experiment may also be applied to a different strain of mouse that is known to eliminate G. muris, for example, the BALB/c mouse.

To test the hypothesis that the cell type crucial for elimination of G. muris is plastic-adherent, lethally irradiated mice should be reconstituted with non-adherent cells. If this hypothesis is correct, the reconstituted mice will develop chronic giardiasis

To test the hypothesis that Lyl+ B cells are crucial for elimination of G. muris, lethally irradiated mice should be reconstituted with resident, peritoneal exudate cells from CBA/Ca mice The peritoneal cavity is a rich source of Lyl+ B cells in adult mice (Havakawa et al 1986). This reconstitution may have to be supplemented with spleen cells or bone marrow cells from CBA/N mice, to reconstitute other hematopoietic cells This supplement should not interfere with the experiment, since CBA/N spleen cells fail to reconstitute the ability of lethally irradiated mice to eliminate G. muris If CBA/Ca peritoneal cells are successful in transferring the ability to eliminate G. muris then Lyl+ B cells should be purified from this source and used to reconstitute lethally irradiated CBA/N mice

3.2 Passive Transfer of Serum, and Immunization of CBA/N Mice to Study the Effect of IgG Antibody on Elimination of G. muris

The purpose of these experiments was to study the possible requirement for IgG antibody in elimination of G. muris. It was previously shown that CBA/N mice made quantitatively deficient serum IgG antibody in response to infection with G. muris, relative to BALB/c mice (Snider, Skea and Underdown 1988). Also, (CBA/N X BALB/c) F1 male mice, which are hemizygous for Xid and failed to eliminate G. muris, made less serum IgG anti-G. muris antibody than (BALB/c X CBA/N) F1 male mice, which do not carry the Xid gene and eliminated G. muris (Snider, Skea and Underdown 1988). Thus, in these experiments, chronic giardiasis in Xid bearing mice was associated with reduced levels of serum IgG anti-G. muris antibody.

This deficiency in the serum IgG anti-G. muris antibody response of CBA/N mice may represent a subclass deficiency. CBA/N mice have a gross deficit in total serum IgG3 (Perlmutter et al. 1978; Eldridge, Meulbroek and McGhee 1988), and their antibody responses are particularly defective in this subclass (Reale, Bona and Schulman 1981; Slack et al. 1980). Thus, the absence of IgG3 antibody may account for the reduced levels of IgG anti-G. muris antibody produced by CBA/N mice

Alternatively, the deficiency in the serum IgG anti-G. muris antibody response of CBA/N mice may represent a specificity defect. CBA/N mice are known to be poorly responsive to certain types of antigens, especially type II T-independent antigens (Scher, Frantz and Steinberg 1973; Scher et al. 1975a; Scher, Berning and Asofsky 1979). Thus, the absence of some specific IgG antibodies may account for the reduced levels of IgG anti-G. muris antibody produced by CBA/N mice. It is also possible that the subclass and specificity defects may be linked. For instance, it is known that type II T-independent antigens tend to stimulate IgG3 antibody responses in immunocompetent mice (Slack et al. 1980). Thus, the immunoglobulin subclass and specificity defects of CBA/N mice may be interdependent, and may both be responsible for the serum IgG anti-G. muris antibody deficiency observed in these mice.

The association between chronic giardiasis in Xid-bearing mice and the deficiency in serum IgG anti-G. muris antibody production by these mice, led to consideration of the hypothesis that the defect of CBA/N mice that renders them susceptible to chronic giardiasis is their deficient serum IgG antibody response. This hypothesis proposes a role for IgG antibody in the elimination of G. muris. For IgG antibody to participate in this process it most likely has to be at the location of the parasite that is, in the intestine. The presence of IgG anti-G. muris antibody in the intestine of G. muris-infected mice is controversial. Snider and Underdown (1986) used a sensitive immunoradiometric assay and failed to detect IgG anti-G. muris antibody in intestinal secretions of G. muris infected BALB/c mice. However, Heyworth (1984) conducted immuno

fluorescence on trophozoites obtained from the intestines of infected, BALB/c mice, and detected IgG antibody bound to the parasite surface.

To test the hypothesis that the defective serum IgG anti-G. muris antibody response of CBA/N mice renders them susceptible to chronic giardiasis, CBA/N mice were provided with serum IgG anti-G. muris antibody. This was accomplished by two methods: 1) active, parenteral immunization of CBA/N mice with G. muris trophozoites and Freund's complete adjuvant, and 2) passive transfer of hyperimmune, anti-G. muris antisera from CBA/J mice to CBA/N mice. The effect of the presence of this antibody on the course of G. muris infection was investigated

3.2.1 Results

3.2.1.1 Comparison of the IgG Antibody Response to G. muris Infection in CBA/N and CBA/Ca Mice

First, the defective serum IgG anti-G. muris antibody response of CBA/N mice was re-examined by comparing the levels of serum IgG anti-G. muris antibody in age-matched, CBA/N and CBA/Ca mice, that had been infected with G. muris for 15 weeks. These data, derived from six separate experiments are shown in Table 5. In five experiments, the CBA/N mice had lower levels of serum IgG anti-G. muris antibody, compared to the CBA/Ca mice. However, this difference was statistically significant in only one of six experiments. Moreover, in one experiment, there was no difference in the levels of this antibody between the two strains.

3.2.1.2 Active Immunization of CBA/N and CBA/Ca Mice with G. muris Trophozoites

Groups of CBA/N and CBA/Ca mice were immunized intraperitoneally with 0.5×10^6 G. muris trophozoites in Freund's complete adjuvant (see 2.9). Each "trophozoite-immunized" mouse received two such injections thirty days apart. Control "sham-immunized" mice received injections of PBS in Freund's complete adjuvant. All of the mice were infected with G.

TABLE 5

SERUM IgG ANTIBODY RESPONSE OF CBA/N AND CBA/Ca MICE TO INFECTION WITH G.MURIS

EXPERIMENT[a]	SERUM IgG ANTI-G.MURIS ANTIBODY (arbitrary units/mL)[b] CBA/N	CBA/Ca
1	18 (16-19)[c]	47[d] (44-50)[c]
2	67 (67-68)	94[e] (81-111)
3	115 (103-127)	113[e] (94-136)
4	171 (150-196)	272[e](150-295)
5	67 (62-73)	93[e] (81-107)
6	103 (96-109)	123[e](114-133)

a In each experiment, CBA/N and CBA/Ca mice were infected with G. muris The mice were bled at 15 weeks post-infection.

b Geometric mean of 5 mice.

c Range of SEM.

d Significantly different from CBA/N, $p<0.01$

e Not significantly different from CBA/N, $p>0.1$

TABLE 6

SERUM IgG ANTI-*G. MURIS* ANTIBODY LEVELS IN ACTIVELY AND PASSIVELY IMMUNIZED CBA/N MICE

EXPERIMENT	MICE	IMMUNIZATION	SERUM IgG ANTI-*G.MURIS* ANTIBODY (arbitrary units/mL)[c]
ACTIVE[a] IMMUNIZATION	CBA/N	TROPHOZOITE	1516[e,g] (1305-1762)[d]
	CBA/J	TROPHOZOITE	3088[g] (2455-3884)
	CBA/N	SHAM	210 (183-242)
	CBA/J	SHAM	289 (274-305)
PASSIVE[b] IMMUNIZATION	CBA/N	CBA/N ANTISERUM	471[f,g] (437-507)
	CBA/N	CBA/J ANTISERUM	1906[g] (1620-2241)
	CBA/N	NONE	67 (62-73)
	CBA/Ca	NONE	93 (81-107)

a Active immunization: Mice received 2 intraperitoneal injections of $0.5X10^6$ trophozoites in Freund's Complete Adjuvant (FCA) (TROPHOZOITE), or FCA alone (SHAM), and were infected 1 week after the last injection. Sera were collected at 4 weeks post-infection.

b Passive immunization: Mice received intraperitoneal injections of 0.2 mL of hyperimmune anti-*G. muris* antiserum prepared as described (see 2.8) in CBA/J or CBA/N mice. Mice were injected every 5 days starting 12 days prior to infection and continuing throughout the infection. Sera were collected at 12 weeks post-infection.

c Geometric mean of 5 mice.

d Range of SEM.

e Not significantly different from actively immunized CBA/J mice, $p>0.1$

f Significantly different from mice passively immunized with CBA/J antiserum, $p<0.01$.

g Significantly different from CBA/N and CBA/J or CBA/Ca controls, $p<0.01$.

muris one week after the second injection. Four weeks later, the mice were bled from the orbital sinus, and levels of serum IgG anti-G. muris antibody were determined. These data are shown in Table 6. The trophozoite-immunized CBA/N mice made a substantial serum IgG antibody response. Although this response was considerably less than that of the trophozoite-immunized CBA/J mice, this difference was not statistically significant, owing to large variations in the responses of individual mice. Nevertheless, the trophozoite-immunized CBA/N mice possessed significantly more serum IgG anti-G. muris antibody than the sham-immunized CBA/J mice.

The course of G. muris infections in the trophozoite-immunized and the sham-immunized mice are shown in Figure 5. Both the trophozoite-immunized and the sham-immunized CBA/N mice developed chronic giardiasis. The trophozoite-immunized and the sham-immunized CBA/J mice eliminated the parasite in an indistinguishable manner. These results were reproduced in two other separate experiments.

3.2.1.3 Passive Transfer of Hyperimmune Anti-G. muris Antisera from CBA/J Mice to CBA/N Mice

Large pools of hyperimmune, anti-G. muris antisera were prepared by injecting 0.5×10^6 G. muris trophozoites, in Freund's complete adjuvant, intraperitoneally into groups of CBA/N and CBA/J mice. Each mouse received two such injections, thirty days apart. The mice were bled from the orbital sinus, 8, 18 and 30 days after the second injection, and were terminally bled from the axillary vein 8 days after the last orbital

bleed. The sera were pooled, according to strain, prior to use. The CBA/J antiserum pool contained approximately three times as much IgG anti-G. muris antibody (10,000 arbitrary antibody units per mL) as the CBA/N antiserum pool (3,450 arbitrary antibody units per mL). These antisera were used to passively transfer serum IgG anti-G. muris antibody to CBA/N mice.

CBA/N mice received intraperitoneal injections of 0.2 mL of hyperimmune, anti-G. muris antisera, each. Since the half-life of mouse IgG is approximately 4.5 days (Waldmann and Strober 1969), the injections were repeated every 5 days. The series of injections was begun 12 days before the mice were infected with G. muris, and was continued throughout the course of infection. The mice received a total of 20 injections over a period of 95 days. Two days after the last injection (12 weeks post-infection), the mice were bled and the levels of serum IgG anti-G. muris antibody in the recipient mice, and in control CBA/N and CBA/Ca mice, that had not received injections, were determined. These data are shown in Table 6. The CBA/N mice that had received injections of CBA/J, hyperimmune, anti-G. muris antiserum had considerable amounts of serum IgG anti-G. muris antibody. The CBA/N mice that had received injections of CBA/N, hyperimmune, anti-G. muris antiserum also had high levels of serum IgG anti-G. muris antibody. However, these mice had significantly less serum IgG anti-G. muris antibody than the mice that had received the CBA/J antiserum. Nevertheless, both groups of mice had significantly more serum IgG anti-G. muris antibody than the control CBA/N and CBA/Ca mice, that had not received injections of antisera.

The course of G. muris infections in mice that received injections of antisera, and those that did not, is shown in Figure 6. The CBA/N mice that received injections of CBA/J or CBA/N, hyperimmune, anti-G. muris antisera, developed chronic giardiasis, as did CBA/N mice that had not received injections of antisera. CBA/Ca mice, which did not receive injections of antisera, eliminated the parasite. These results were reproduced in one other separate experiment.

Figure 5

CBA/N mice (●) and CBA/J mice (■) were immunized by 2 intraperitoneal injections of $5X10^5$ G. muris trophozoites in Freund's complete adjuvant (0.4 mL of a 1:1 antigen:adjuvant emulsion). The injections were given 4 weeks apart. Control CBA/N mice (○) and CBA/J mice (□) were sham-immunized by a similar procedure, except that the trophozoite suspension was replaced with an equal volume of PBS. One week after the final injection (at week 0), the mice were infected by peroral administration of 5000 G. muris cysts. The course of infection was followed by measuring cyst output in feces. Each point represents the mean of values from 5 mice. Error bars represent the standard error of the mean. The arrow marks the limit of detection (600 cysts per 8 fecal pellets).

IMMUNIZATION OF MICE WITH G. MURIS TROPHOZOITES: COURSE OF G. MURIS INFECTION IN IMMUNIZED AND SHAM-IMMUNIZED MICE

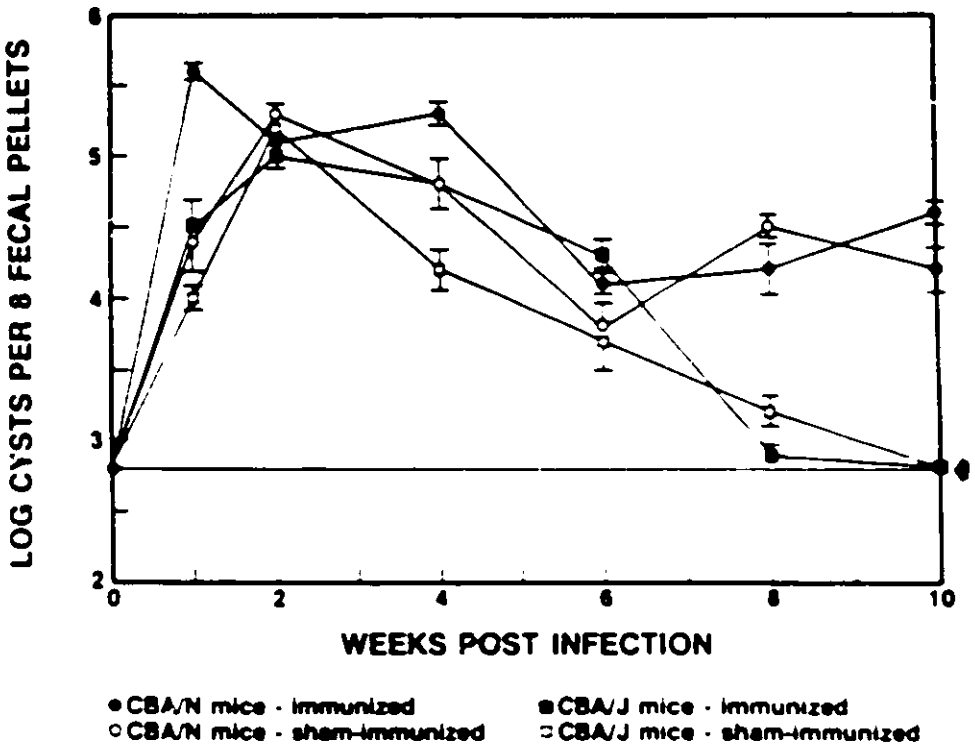
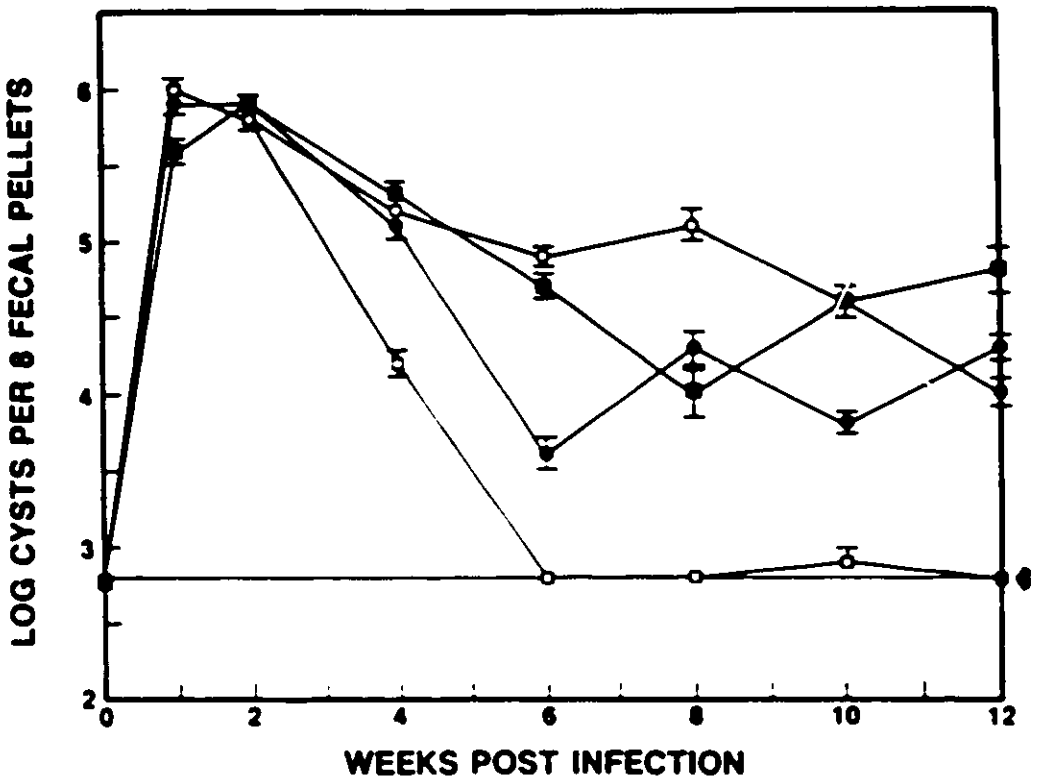


Figure 6

Hyperimmune anti-G. muris antisera were prepared from CBA/N and CBA/J mice that had been immunized by 2 intraperitoneal injections of $5X10^5$ G. muris trophozoites in Freund's complete adjuvant. These antisera were injected into recipient CBA/N mice as follows -- 0.2 mL of CBA/N hyperimmune anti-G. muris antiserum (●), or 0.2 mL of CBA/Ca hyperimmune anti-G. muris antiserum (■), was injected, intraperitoneally, into CBA/N mice, every 5 days, beginning 12 days prior to G. muris infection, and continuing throughout the course of the infection. Intact CBA/N (○) and CBA/Ca (□) mice received no injections over the course of their infections. Infection in the mice was initiated (= week 0) by peroral administration of 5000 G. muris cysts. The course of infection was followed by measuring cyst output in feces. Each point represents the mean of values from 5 mice. Error bars represent the standard error of the mean. The arrow marks the limit of detection (600 cysts per 8 fecal pellets).

PASSIVE TRANSFER OF HYPERIMMUNE ANTI-G. MURIS ANTISERA TO CBA/N MICE: COURSE OF G. MURIS INFECTION IN RECIPIENT MICE



● CBA/N mice - received CBA/N hyperimmune anti-G.muris antiserum
■ CBA/N mice - received CBA/J hyperimmune anti-G.muris antiserum
○ CBA/N mice
▽ CBA/Ca mice

3.2.2 Discussion

It was previously reported that chronic giardiasis in CBA/N mice was associated with reduced levels of serum IgG antibody against the parasite (Snider, Skea and Underdown 1988). This observation led to consideration of the hypothesis that CBA/N mice fail to eliminate G. muris because they have insufficient or inappropriate serum IgG anti-G. muris antibody. However, upon re-examination, only small differences were found between the serum IgG anti-G. muris antibody responses of CBA/N mice and CBA/Ca mice. In five of six experiments, CBA/N mice made slightly less serum IgG antibody, than CBA/Ca mice, in response to infection with G. muris (Table 5). In addition, in two experiments, CBA/N mice made less serum IgG antibody than CBA/Ca mice, in a secondary response to parenteral immunization with G. muris trophozoites (Table 6). Thus, there may be a small defect in the ability of CBA/N mice to synthesize serum IgG anti-G. muris antibody. However, this defect is not as prominent as previously described (Snider, Skea and Underdown 1988). In the present study the differences in the levels of serum IgG anti-G. muris antibody in CBA/N mice and CBA/Ca mice were not always statistically significant, and were not invariably reproducible. The apparent discrepancy between this study and the previous one may be attributed to the strain of mice used as a basis for comparison. The previous study compared CBA/N mice to BALB/c

mice, and (CBA/N X BALB/c) F1 male mice to (BALB/c X CBA/N) F1 male mice. Genetic factors other than Xid may contribute to the immune response against G. muris. An ideal study would compare the serum IgG anti-G. muris antibody responses of mice congenic for Xid.

In spite of its limitations, the hypothesis that chronic giardiasis in CBA/N mice is due to insufficient serum IgG anti-G. muris antibody production, was tested by providing CBA/N mice with this antibody, and examining its effect on the course of G. muris infection.

Active, parenteral immunization of CBA/N mice and CBA/J mice, with G. muris trophozoites in Freund's complete adjuvant, did not alter the course of subsequent G. muris infection in either strain (Figure 5). Although trophozoite-immunized CBA/N mice possessed more serum IgG anti-G. muris antibody than sham-immunized CBA/J mice (Table 6), the former mice developed chronic giardiasis, while the latter mice eliminated the parasite. Thus, in this experiment, chronic giardiasis was not associated with reduced levels of serum IgG anti-G. muris antibody.

Prior, parenteral immunization with trophozoites offered no degree of protection against G. muris infection, in either strain of mice. This result is consistent with previous results obtained by Erlich et al (1983). These investigators found that parenteral immunization of BALB/c and C3H/He mice, with whole or sonicated trophozoites, failed to protect the mice against subsequent G. muris infection. This group had previously reported a protective effect in BALB/c mice (Roberts-Thomson and Mitchell 1979), but were subsequently unable to reproduce this result (Erlich et al. 1983). The failure of parenteral immunization to protect against an intestinal infection is not surprising. Protection against an intestinal

infection most likely requires stimulation of memory in the mucosal immune system rather than in the systemic immune system (Befus and Bienenstock 1980).

Although trophozoite-immunized CBA/N mice possessed large quantities of serum IgG anti-G. muris antibody, they still may have lacked the correct subclass or specificity of IgG antibody, that may be required for elimination of G. muris. A passive transfer experiment was conducted to test this hypothesis.

The abilities of CBA/N and CBA/J hyperimmune anti-G. muris antisera to passively transfer, to CBA/N mice, the ability to eliminate G. muris, were compared. In this respect, there was no difference between the two antisera; neither had any effect on the course of G. muris infection in CBA/N mice (Figure 6). The elevated levels of IgG anti-G. muris antibody in the sera of the recipient mice demonstrated the success of the antibody transfer, and reflected the differences in the amounts of IgG anti-G. muris antibody present in the CBA/N and CBA/J donor sera (Table 6). Although the CBA/N recipients of hyperimmune antisera possessed more serum IgG anti-G. muris antibody than control CBA/Ca mice, which did not receive serum, the former mice developed chronic giardiasis, while the latter mice eliminated the parasite. Thus, in this experiment also, chronic giardiasis was not associated with reduced levels of serum IgG anti-G. muris antibody.

The failure of passively transferred serum antibody to alter the course of G. muris infection in recipient mice is consistent with the previous observations of Erlich et al. (1983). This group found that passive immunization of BALB/c mice with sera from G. muris-immune BALB/c

mice, did not confer any degree of resistance to G. muris infection. Butscher and Faubert (1988) reported that intraperitoneal injection of a monoclonal IgM anti-G. muris antibody offered partial protection against G. muris infection in BALB/c mice. This conclusion was based on the observation that the recipient mice had an average of 66% fewer trophozoites in the intestine, relative to control mice, at one time point. This claim was not confirmed by examining trophozoite burden or cyst output at other time points during the infection. Thus, the possible protective effect of this antibody remains to be verified.

The failure of passively transferred serum antibody to affect G. muris infection, is most likely due to its failure to reach the intestine. Neonatal, suckling mice were protected against G. muris infection by passive transfer of immune milk containing IgA and IgG antibodies (Andrews and Hewlett 1981). In addition, mice infected with G. muris by direct transfer of trophozoites to the intestine (by laparotomy), were partially protected when the transferred parasites were pre-incubated with polymeric IgA and IgM antibody (Underdown et al. 1988). Thus, passive immunization against G. muris infection can be effective, but only when the transferred antibody is able to reach the intestine.

It seemed unlikely that serum IgG antibody would be important for the elimination of an intestinal parasite However, since chronic giardiasis in CBA/N mice was associated with reduced levels of serum IgG anti-G. muris antibody (Snider, Skea and Underdown 1988), this possibility was investigated. In the present series of experiments, the association between chronic giardiasis and reduced levels of serum IgG anti-G. muris antibody was not upheld. The provision of CBA/N mice with serum IgG anti-

G. muris antibody, by active immunization or by passive transfer, did not alter their susceptibility to chronic giardiasis. CBA/N mice that possessed large quantities of serum IgG anti-G. muris antibody still failed to eliminate the parasite. Therefore, the hypothesis was rejected, and the conclusion was made, that serum IgG antibody is not a crucial element in the elimination of G. muris.

3.3 Analysis of G. muris Antigen Recognition by Antibodies from CBA/N Mice

CBA/N mice are known to be poorly responsive to some antigens and non-responsive to others (Quintans and Kaplan 1978; Scher et al. 1975b). Thus, I considered the hypothesis that CBA/N mice fail to eliminate G. muris because they fail to recognize a crucial antigen or antigens on the parasite that stimulate immune elimination in other strains of mice. Such an antigen would be considered a host-protective antigen, and its identification would provide a potential candidate for a subunit vaccine.

In a previous study, Erlich et al. (1983) examined a similar hypothesis, that C3H/He mice fail to eliminate G. muris because they fail to recognize a crucial antigen. These investigators identified an 82K protein and a complex of four acidic, 32K, proteins that were better recognized by antibodies from immunized and infected BALB/c (which eliminate G.muris), than by antibodies from immunized and infected C3H/He mice. Thus, susceptibility to chronic giardiasis in C3H/He mice was associated with poor responsiveness to certain parasite antigens. However, this association was not sustained upon examination of (BALB/c X C3H/He) F2 mice. Poor responsiveness to these antigens did not segregate with susceptibility to chronic giardiasis; some of the F2 mice recognized these antigens, but failed to eliminate G. muris.

The purpose of the following experiments was to attempt to identify an antigen or antigens of G. muris that are recognized by antibodies from CBA/Ca or CBA/J mice, but not by antibodies from CBA/N mice. The Western blot technique was used to analyze recognition of protein and glycoprotein antigens of G. muris trophozoites and cysts. In addition, recognition of lipid and glycolipid antigens of G. muris trophozoites was investigated by organic solvent extraction, dot blot analysis and thin layer chromatography. Isotype-specific antibody-enzyme conjugates were used to examine antigen binding by serum IgG, IgA and IgM antibodies.

3.3.1 Results

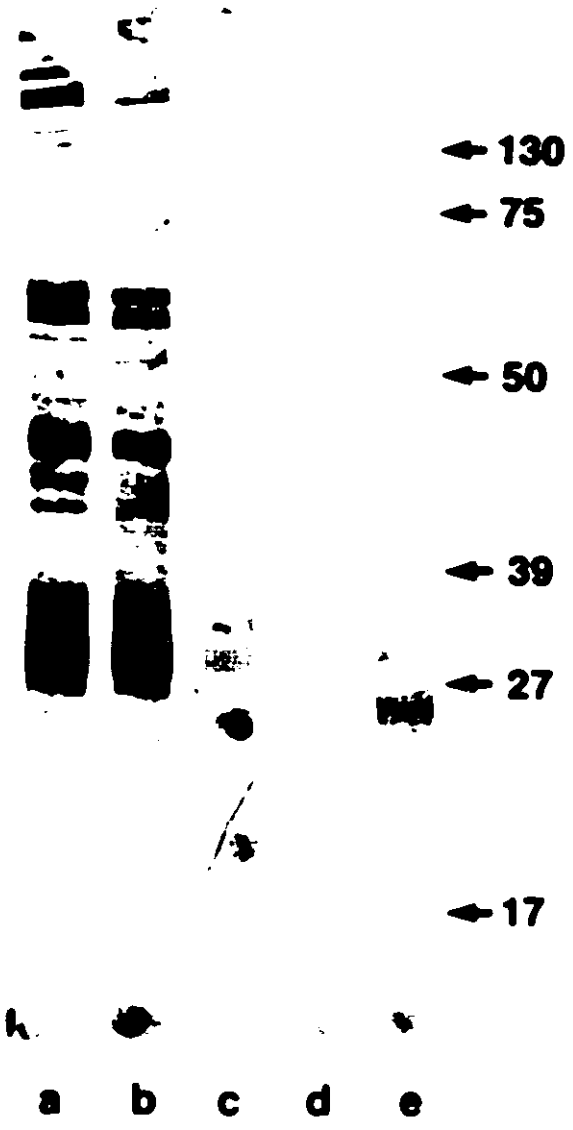
3.3.1.1 Western Blot Analysis of G. muris Protein and Glycoprotein Antigens

The ability of CBA/N mice to respond to G. muris trophozoite antigens was examined by immunizing and boosting CBA/N and CBA/J mice, intraperitoneally, with optimal, immunogenic doses of G. muris trophozoites in Freund's complete adjuvant (see 2.8). Antigen recognition by the hyperimmune antisera thus produced, was analyzed using the Western blot technique (see 2.14). Briefly, G. muris trophozoite antigens were electrophoretically separated on a 10% SDS-polyacrylamide gel and transferred to nitrocellulose. Pooled hyperimmune antisera from 30 mice of each strain were incubated, at a dilution of 1:100, with the antigen-coated nitrocellulose strips. Pooled sera from 10 age-matched, non-immunized mice of each strain (normal sera) were used at the same dilution. Antibody binding to the G.muris trophozoite antigens was visualized by incubation of the blots with the antibody-enzyme conjugate, goat anti-mouse IgG-alkaline phosphatase, followed by the enzyme-catalyzed colour development reaction. A representative blot is shown in Figure 7 The antigens recognized by the hyperimmune antisera included major bands with approximate molecular weights (MW) of 30K, 45K, 60K and 65K. Some

Figure 7

G. muris trophozoite antigens were separated on a 10% SDS-polyacrylamide gel and transferred to nitrocellulose. After blocking with 5% normal goat serum in PBS (NGS-PBS), the blots were incubated with 1:100 dilutions of mouse sera in NGS-PBS. The sera were from (a) CBA/J mice, immunized with G. muris trophozoites, (b) CBA/N mice, immunized with G. muris trophozoites, (c) age-matched, non-immunized CBA/J mice, and (d) age-matched, non-immunized CBA/N mice. Lane (e) was incubated with NGS-PBS alone. Following the incubation, the blots were washed and incubated with goat anti-mouse IgG-alkaline phosphatase. Antibody binding was visualized by performing the alkaline phosphatase colour development reaction (see 2.14).

WESTERN BLOT ANALYSIS OF G. MURIS TROPHOZOITE ANTIGENS RECOGNIZED BY HYPERIMMUNE ANTI - G. MURIS ANTISERA FROM CBA/N AND CBA/J MICE



high molecular (>130K) antigens were also recognized. In addition, a number of less intense bands, at various MWs, were observed. There were no major differences in G. muris trophozoite antigen recognition between the CBA/N and the CBA/J hyperimmune antisera. There were small differences in the intensity of some bands. However, all antigens that were recognized by the CBA/J hyperimmune antiserum were also recognized by the CBA/N hyperimmune antiserum. No antigens were recognized by the normal sera from non-immunized mice, at this dilution.

The serum antibody response of mice undergoing a natural infection with G. muris was also studied using the Western blot technique. Pooled antisera from 10 CBA/N mice and from 10 CBA/Ca mice, that had been infected with G. muris for 12 weeks, were used at a dilution of 1:25. Pooled sera from 10 age-matched, non-infected mice of each strain (normal sera) were used at the same concentration. Recognition of G. muris trophozoite and cyst antigens by serum IgG, IgA and IgM antibodies was examined using isotype-specific, antibody-enzyme conjugates. Representative blots are shown in Figures 8 (IgG antibody), 9 (IgA antibody) and 10 (IgM antibody).

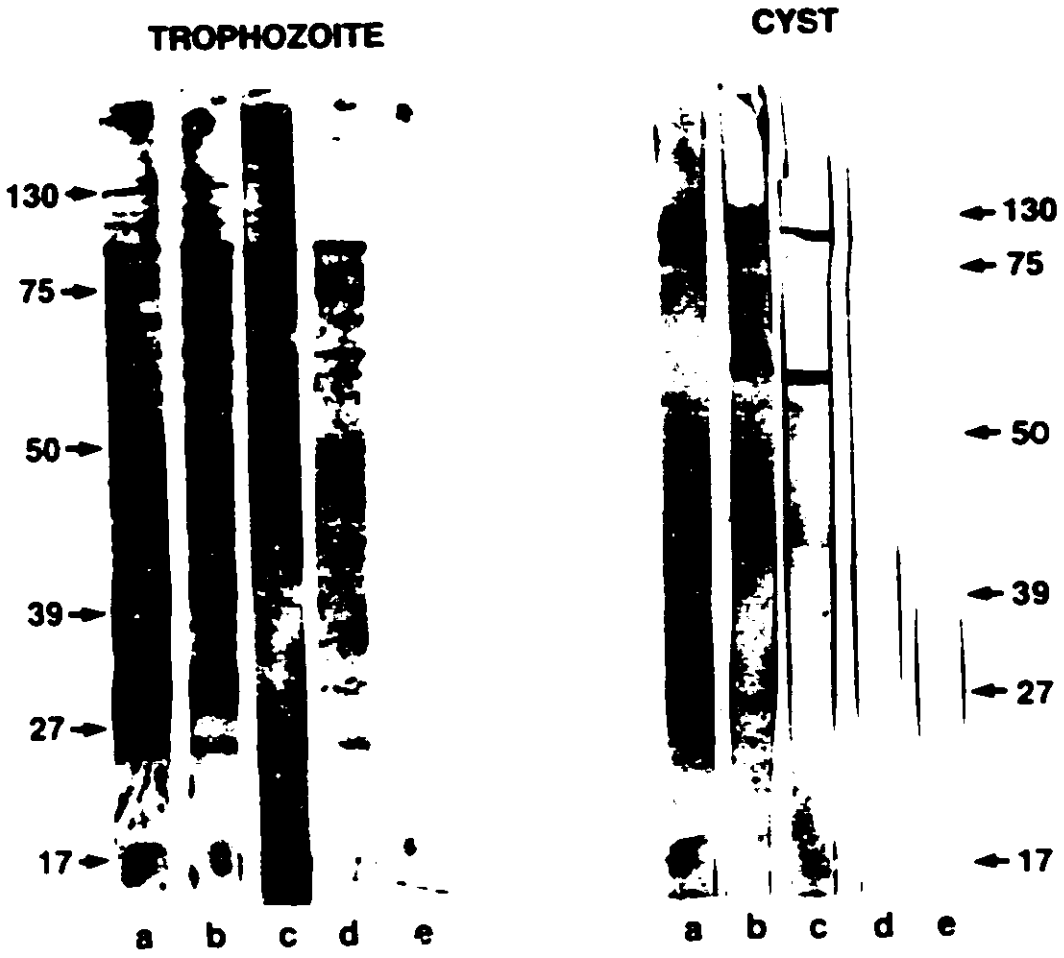
The G. muris trophozoite antigen recognition profile of serum IgG antibodies from infected mice (Figure 8) was slightly different from that of serum IgG antibodies from immunized mice (Figure 7). In both cases, the 30K, 60K and 65K trophozoite antigens were recognized. However, serum IgG antibodies from infected mice additionally recognized antigens of MWs 40K, 70K and 80K, but did not recognize the 45K antigen or the high MW (>130K) antigens. Normal mouse sera IgG also reacted with the 65K and 80K antigens, at the lower dilution. This reaction may represent some natural

Figures 8, 9 and 10

G. muris trophozoite and cyst antigens were separated on a 10% SDS-polyacrylamide gel and transferred to nitrocellulose. After blocking with 5% normal goat serum in PBS (NGS-PBS), the blots were incubated with 1:25 dilutions of mouse sera. The sera were from (a) CBA/Ca mice, infected for 12 weeks with *G. muris*, (b) CBA/N mice, infected for 12 weeks for *G. muris*, (c) age-matched, non-infected CBA/Ca mice and (d) age-matched, non-infected CBA/N mice. Lanes (e) were incubated with NGS-PBS alone. Following this incubation, the blots were washed and incubated with (Figure 8) goat anti-mouse IgG-alkaline phosphatase, (Figure 9) goat anti-mouse IgA-alkaline phosphatase, or (Figure 10) goat anti-mouse IgM-alkaline phosphatase. Antibody binding was visualized by performing the alkaline phosphatase colour development reaction (see 2.14).

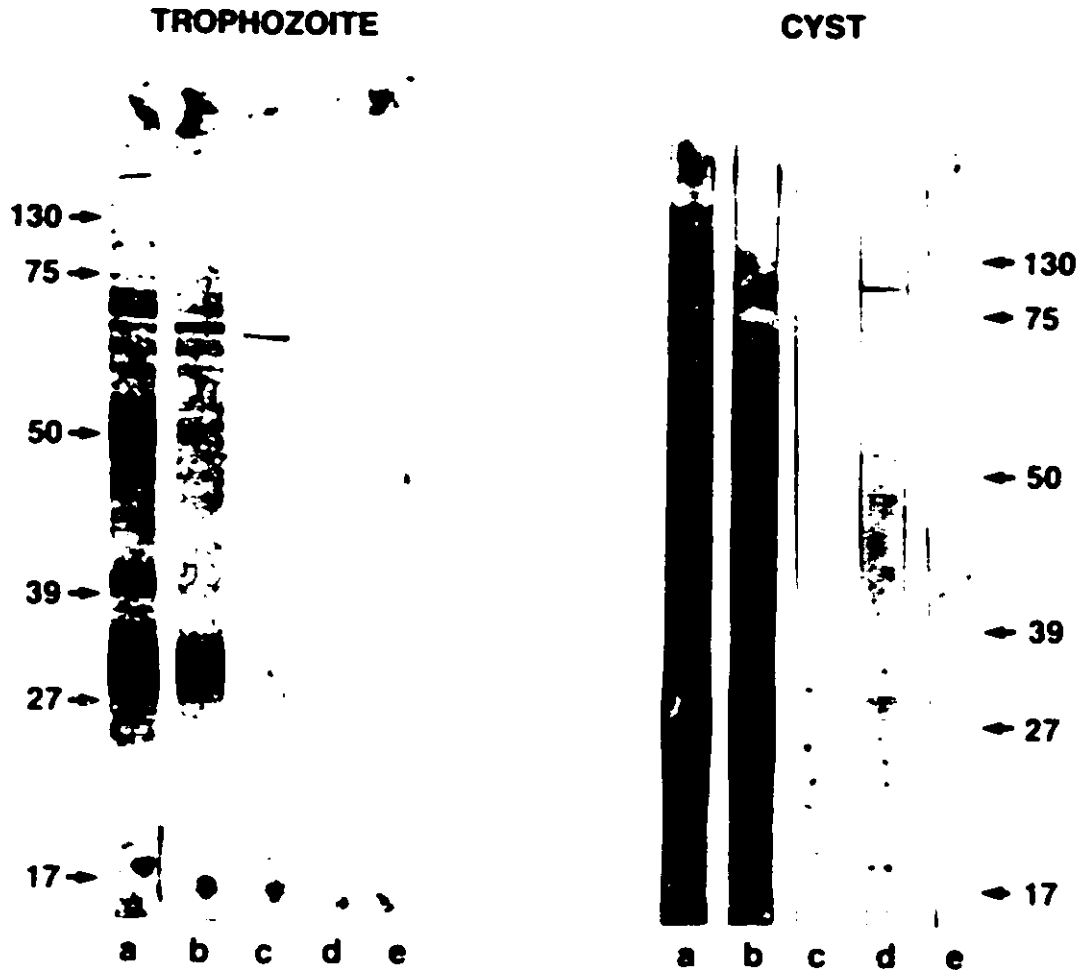
8

WESTERN BLOT ANALYSIS OF G. MURIS TROPHOZOITE AND CYST ANTIGENS RECOGNIZED BY SERUM IgG ANTIBODIES FROM G. MURIS - INFECTED CBA/N AND CBA/Ca MICE



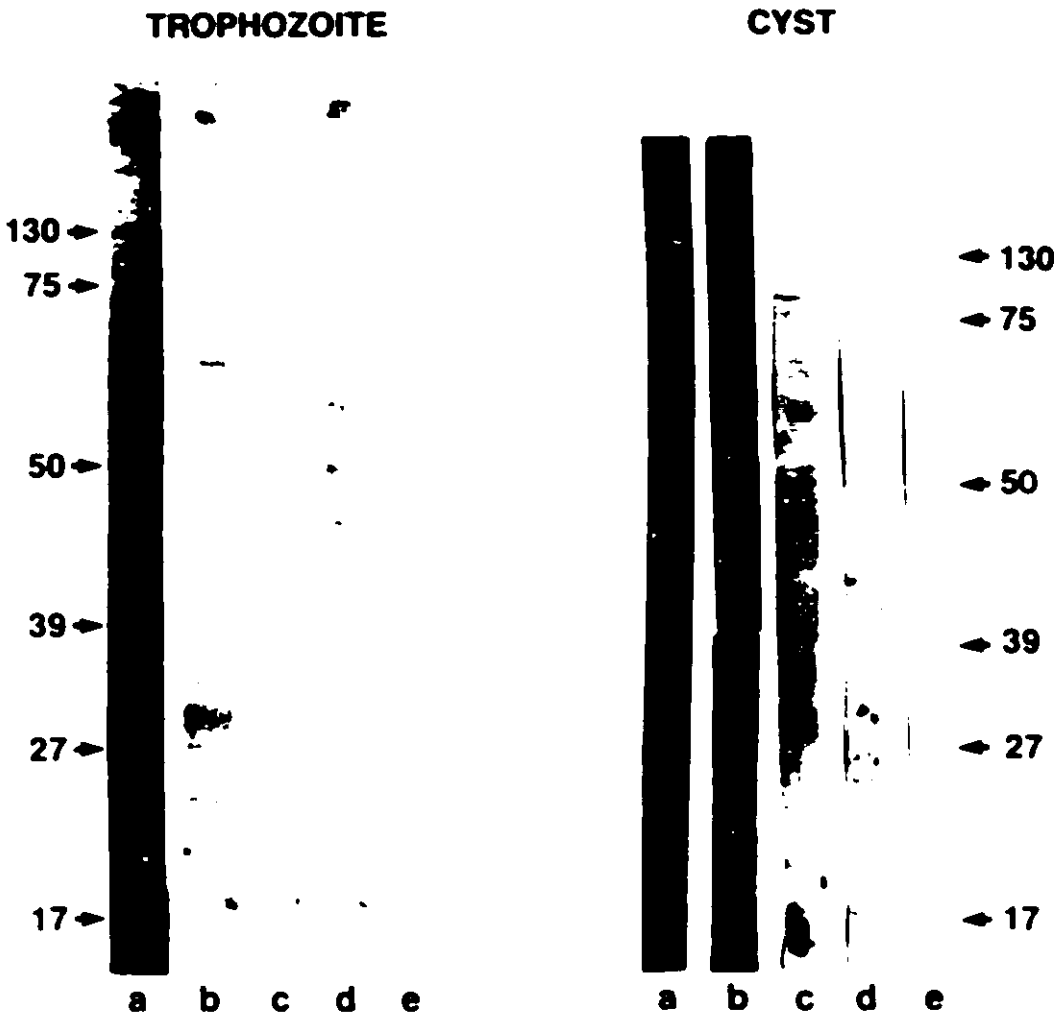
9

WESTERN BLOT ANALYSIS OF G. MURIS TROPHOZOITE AND CYST ANTIGENS RECOGNIZED BY SERUM IgA ANTIBODIES FROM G. MURIS - INFECTED CBA/N AND CBA/Ca MICE



10

WESTERN BLOT ANALYSIS OF G. MURIS TROPHOZOITE AND CYST ANTIGENS RECOGNIZED BY SERUM IgM ANTIBODIES FROM G. MURIS - INFECTED CBA/N AND CBA/Ca MICE



antibody to the parasite. Alternatively, it may represent some, non-specific binding of mouse IgG by the parasite molecules. Nonetheless, the reactions of these antigens, with sera from infected mice, were more intense than those with sera from non-infected mice. Thus, these antigens were immunogenic in G. muris-infected mice.

Serum IgG antibodies from infected mice recognized a 100K G. muris cyst antigen (Figure 8). This band was also faintly visible in the normal sera lanes, and in the lane that was not exposed to mouse sera. These faint bands may represent some non-specific binding to the antigen, by the second antibody-enzyme conjugate. The normal CBA/Ca serum showed some binding of IgG to a cyst molecule with an approximate MW of 60K. The reason for this reaction is unknown.

There were no major differences in the G. muris trophozoite or cyst antigen recognition profiles of serum IgG antibodies from infected CBA/N and CBA/Ca mice (Figure 8).

The G. muris trophozoite antigen recognition profile of serum IgA antibodies from infected mice (Figure 9) was similar to that of IgG antibodies (Figure 8). Serum IgA antibodies recognized the 30K, 40K, 60K and 65K trophozoite antigens, but not the 80K antigen. There was some reaction between normal mouse sera IgA and the 65K antigen. This may have been due to some natural IgA antibody to the parasite, or to non-specific binding of mouse IgA by the 65K molecule.

Serum IgA antibodies from infected mice recognized the 100K cyst antigen and additionally, recognized a pair of antigens with slightly smaller MWs (Figure 9). The anti-mouse IgA antibody-enzyme conjugate,

like the anti-mouse IgG antibody-enzyme conjugate, showed some binding to the 100K cyst antigen.

There were no major differences in the G. muris trophozoite or cyst antigen recognition profiles of serum IgA antibodies from infected CBA/N and CBA/Ca mice.

The Western blot of G. muris trophozoite antigens that was developed with the anti-mouse IgM antibody-enzyme conjugate showed faint patterns of bands in all lanes that were exposed to mouse sera (Figure 10). These faint banding patterns may have been due to non-specific sticking of IgM molecules to the trophozoite proteins. Nonetheless, there was a series of bands between 50K and 130K that were darker in the lanes that were exposed to sera from infected mice compared to the lanes that were exposed to sera from normal mice. Some of these bands, in particular the doublet at 60K, were darker in the lane that was exposed to sera from infected CBA/Ca mice compared to the lane that was exposed to sera from infected CBA/N mice. In addition, there was a faint band at approximately 40K, that was visible in the lane that was exposed to sera from infected CBA/Ca mice but not in the other lanes.

Serum IgM antibodies from infected CBA/N and CBA/Ca mice recognized a 55K G. muris cyst antigen (Figure 10). Sera from non-infected mice showed no binding of IgM to G. muris cyst antigens

3.2.1.2 Analysis of G. muris Lipid and Glycolipid Antigens

Lipid components of G. muris trophozoites were extracted using a mixture of chloroform and methanol (see 2.13). The concentrated extract

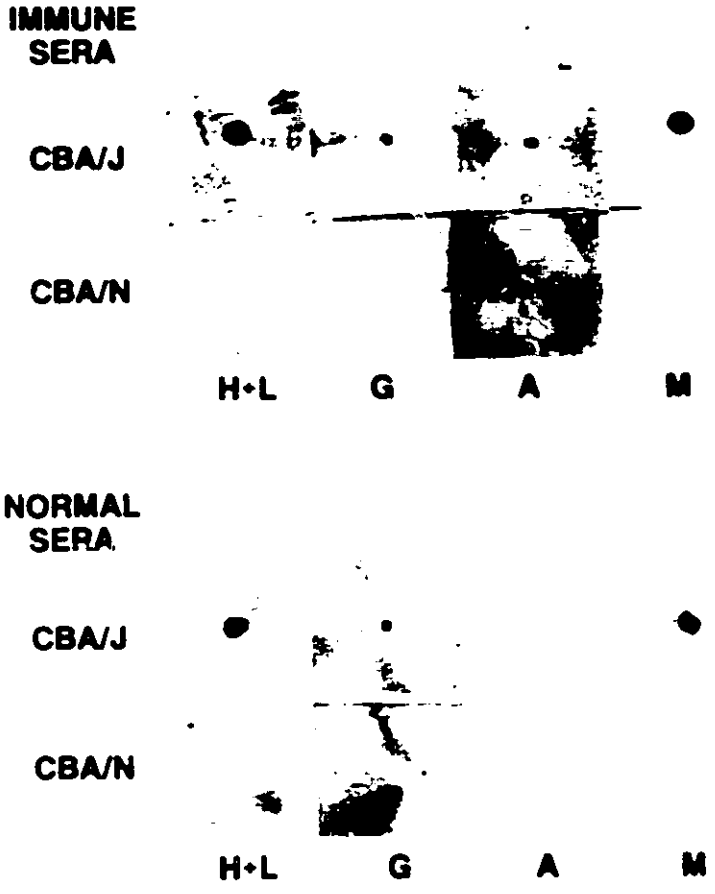
was spotted onto squares of nitrocellulose. Antibody binding to components of the extract was tested using a dot blot procedure. Pooled antisera from 20 CBA/N mice and from 20 CBA/J mice, that had been infected with G. muris for 12 weeks, were used at a dilution of 1:25. Pooled sera from 10 age-matched, non-infected mice of each strain (normal sera) were used at the same concentration. Antibody binding was detected using an antibody-enzyme conjugate specific for mouse immunoglobulin heavy and light chains (H+L). Isotype analysis of antibody binding was conducted using antibody-enzyme conjugates specific for mouse IgG, IgA and IgM. A representative dot blot is shown in Figure 11. Serum immunoglobulin from infected and non-infected CBA/J mice bound to the trophozoite lipid antigen spots. Serum immunoglobulin from infected and non-infected CBA/N mice did not. Isotype analysis revealed that the binding activity in CBA/J sera was due to IgM. IgG and IgA did not bind to the trophozoite lipid antigen spots.

Since serum IgM from both infected and non-infected CBA/J mice bound to the trophozoite lipid antigen spots, this binding activity may represent some natural IgM antibody against a lipid antigen of G. muris trophozoites. The lack of binding activity in the CBA/N sera may represent an absence of this natural antibody. Alternatively, the binding activity in the CBA/J sera may represent a non-immunologic reaction between IgM and a G. muris trophozoite lipid component. The total serum IgM level of CBA/N mice is approximately 20% of that of CBA/J mice. Thus, the observed difference in binding activity, between CBA/N and CBA/J sera, may have been due to the quantitative difference in the levels of IgM. These hypotheses were tested by repeating the dot blot using matched IgM

Figure 11

G. muris trophozoites were extracted with chloroform:methanol:water (3:6:2). The extract was evaporated to dryness, and the residue was redissolved in a small volume of chloroform:methanol (2:1). A total of 0.02 mL of extract, containing $5X10^4$ trophozoite equivalents, was spotted onto each of 16 nitrocellulose squares. The squares were blocked with 5% normal goat serum in PBS, following which, each was incubated with 10 mL of a 1:25 dilution of serum. The sera were derived from CBA/J and CBA/N mice that had been infected with G. muris for 12 weeks (immune sera), or from age-matched, non-infected, control mice (normal sera). Following the incubation, the nitrocellulose squares were washed, and then incubated with "second antibody-horse radish peroxidase (HRP) conjugates"; these conjugates were specific for immunoglobulin heavy and light chains (H+L), for IgG (G), for IgA (A), and for IgM (M). Following this incubation, the nitrocellulose squares were washed, and the HRP-colour development reaction was performed.

DOT BLOT ANALYSIS OF CHLOROFORM:METHANOL EXTRACT OF G. MURIS TROPHOZOITES: INVESTIGATION OF BINDING BY IMMUNOGLOBULIN ISOTYPES FROM CBA/N AND CBA/J MICE



concentrations, rather than matched serum concentrations. The sera were diluted to a final concentration of 10 μg/mL IgM; the CBA/N sera were diluted approximately 1:5, while the CBA/J sera were diluted approximately 1:25. In addition, sera from infected and non-infected BALB/c mice were tested, using the same conditions. Two purified myeloma IgM proteins, originally derived from BALB/c mice, were also tested, at 10 μg/mL. This dot blot is shown in Figure 12. At a constant concentration of 10 μg/mL IgM, the CBA/J and the BALB/c sera showed binding of IgM to the trophozoite lipid antigen spots, as did the two myeloma IgM proteins. However, the CBA/N sera showed no binding activity, under these conditions.

The binding of myeloma IgM proteins to the trophozoite lipid antigen spots supports the hypothesis that the reaction is non-immunologic. The failure of CBA/N IgM to bind to the trophozoite lipid antigen spots, at the same IgM concentration at which CBA/J and BALB/c IgM bound, suggests that there may be a qualitative difference between CBA/N IgM and CBA/J and BALB/c IgM.

Thin layer chromatography was used to identify the trophozoite lipid component that was involved in the binding of IgM. Thin layer chromatograms of G. muris trophozoite lipids were stained with iodine (for total lipids) and orcinol in sulphuric acid (for glycolipids), or were subjected to immunoblotting with sera from infected and non-infected CBA/J mice. These chromatograms are shown in Figure 13. CBA/J serum IgM bound to a glycolipid that had a relative mobility value of 0.45. There was also a small amount of binding of IgM to the more polar lipid components that remained near the origin. This reaction was slightly more intense

with the serum from infected CBA/J mice (immune serum), compared to serum from non-infected CBA/J mice (normal serum).

Figure 12

This experiment was the same as that described in Figure 11, with the following exceptions: (1) all of the sera and the two myeloma proteins (IgMκ and IgMλ) were used at a concentration of 10 μg/mL IgM, and (2) the "second antibody-HRP conjugate" was specific for IgM.

DOT BLOT ANALYSIS OF CHLOROFORM:METHANOL EXTRACT OF G. MURIS TROPHOZOITES: INVESTIGATION OF BINDING BY IgM FROM CBA/N, CBA/J AND BALB/c MICE

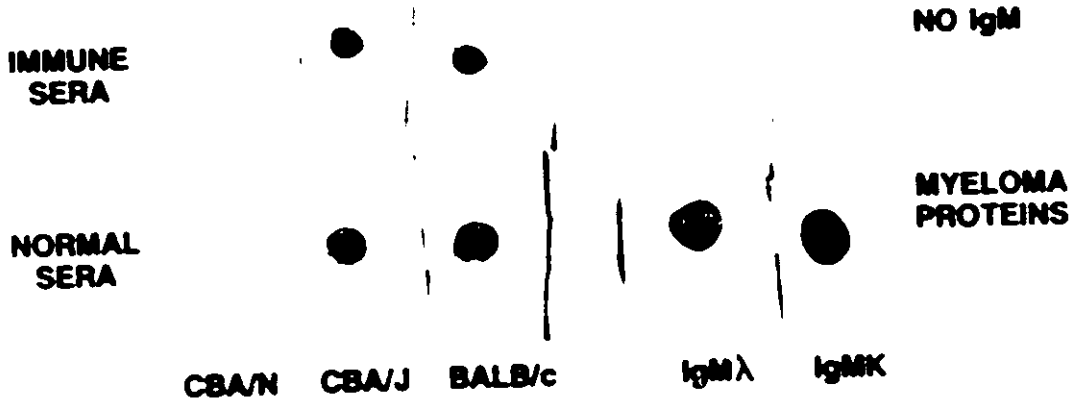


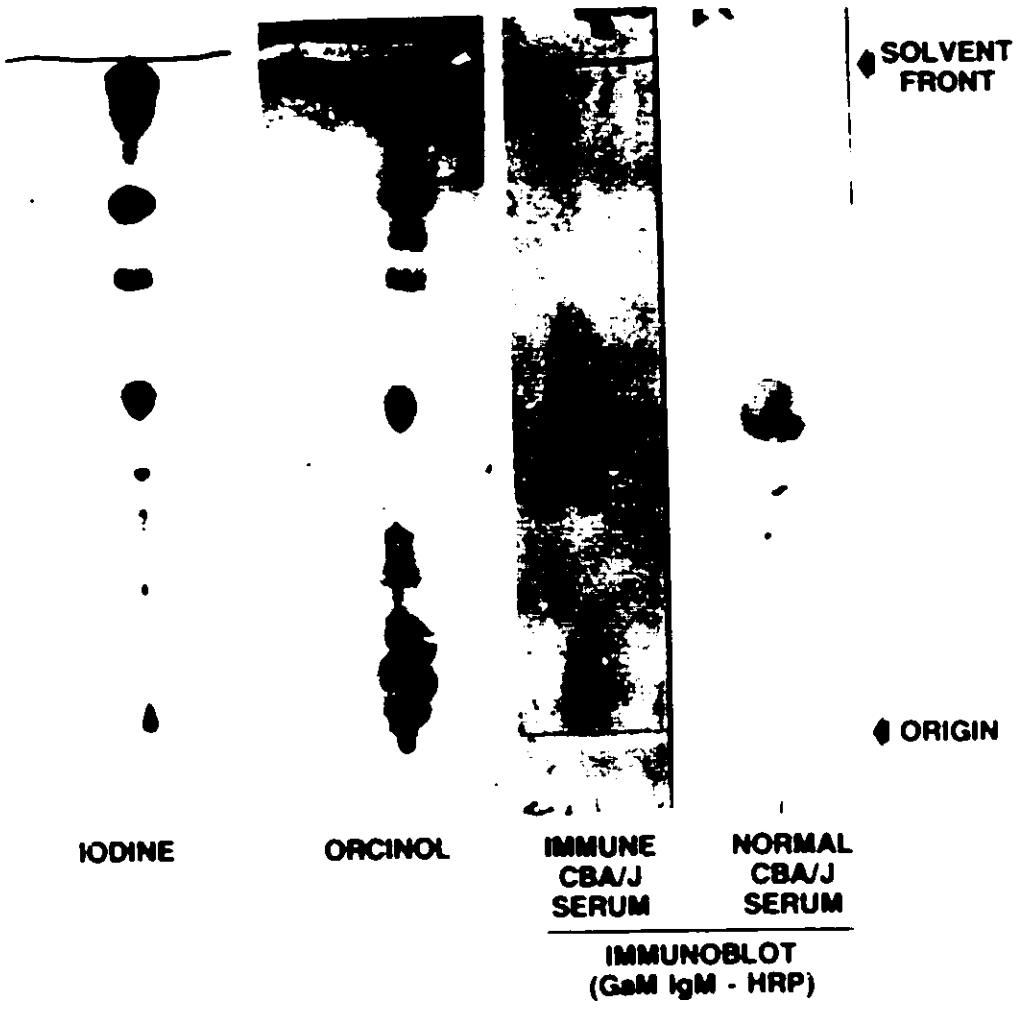
Figure 13

Thin layer chromatography of G. muris lipid extract (described in the legend for Figure 11) was performed using silica gel-coated plates, and the developing solvent system, chloroform:methanol:0.25% potassium chloride (60:35:8).

Samples of 0.05 mL of extract, containing $9X10^6$ trophozoite equivalents, were spotted onto the iodine- and orcinol-stained plates. Following thin layer chromatography, the iodine-stained plate was developed in a tank of iodine vapour until the lipid spots were visible. The orcinol-stained plate was sprayed with a mixture of orcinol, ferric chloride and sulphuric acid, and heated at 100°C for 15 minutes, during which time the glycolipid spots became visible.

For immunoblotting, samples of 0.05 mL of extract, containing $30X10^6$ trophozoite equivalents, were spotted onto the plates. Following chromatography, the plates were treated with the stabilizer, polyisobutylmethacrylate, and then were blocked with 5% normal goat serum in PBS. Each plate was incubated with 30 mL of a 1:25 dilution of serum. The sera used were immune serum from CBA/J mice infected for 12 weeks with G. muris, and normal serum from age-matched, non-infected mice of the same strain. The plates were washed, then incubated with a "second antibody-HRP" conjugate specific for IgM. The plates were washed again, and the HRP-colour development reaction was performed.

THIN LAYER CHROMATOGRAPHY OF CHLOROFORM:METHANOL EXTRACT OF G. MURIS TROPHOZOITES



3.3.2 Discussion

The Western blot analysis of G. muris antigen recognition was conducted to test the hypothesis that CBA/N mice may fail to recognize a crucial antigen on the parasite. It was thought that such a specificity defect in the IgA isotype might have implications for the underlying cause of chronic giardiasis in this strain. Additionally, it was thought that such a specificity defect in the IgG isotype might account for the reduced levels of IgG anti-G. muris antibody produced by CBA/N mice. There were no major differences in the G. muris trophozoite or cyst antigen recognition profiles of serum IgA or IgG antibodies from CBA/N and CBA/Ca mice.

There were some differences in the recognition of trophozoite antigens by IgM antibodies. IgM antibodies from infected CBA/Ca mice recognized a doublet of antigens at 60K and an antigen at 40K. These antigens were not recognized by IgM antibodies from infected CBA/N mice. This result is consistent with previous observations that CBA/N mice are particularly defective in making antibody responses of the IgM isotype (Boswell et al. 1980; Correa-Oliveira and Sher 1985, Brinkmann, Remington and Sharma 1987). However, the observation that CBA/Ca mice made IgM antibody to G. muris trophozoites was unexpected since Snider and Underdown (1986), using a sensitive immunoradiometric assay and sonicated G. muris trophozoites as antigen, failed to detect IgM antibodies in the

sera of infected BALB/c and C57BL/6 mice. The reason for the discrepancy is unknown. It may be due to the difference in mouse strains used to analyze the IgM response. Alternatively, it may be that the antigens described here were inaccessible to IgM antibodies in the sonicated trophozoite antigen preparation used by Snider and Underdown (1986).

Snider and Underdown (1986) also failed to detect IgM anti-G. muris antibodies in concentrated intestinal lavage fluids obtained from infected BALB/c and C57BL/6 mice. In addition, Heyworth (1986) failed to detect, by immunofluorescence, IgM antibodies on the surface of G. muris trophozoites obtained from the intestines of infected BALB/c mice. Since IgM antibody has not been found in the intestine, its importance in the elimination of the parasite is unlikely. Thus, the significance of the finding of differential recognition of trophozoite antigens by serum IgM antibodies from CBA/N and CBA/Ca mice, in terms of elimination of G. muris, remains uncertain.

D. Snider (1985) conducted an analysis of G. muris trophozoite antigen recognition by serum IgG antibodies, and serum and intestinal IgA antibodies. He used surface radiolabelling of G. muris trophozoites and immunoprecipitation techniques to examine the antibody response of CBA/N mice to infection with G. muris. He found no differences in antigen recognition between CBA/N antibodies and CBA/J or BALB/c antibodies, that could be related to susceptibility to chronic giardiasis. He identified a 78K trophozoite surface antigen that was recognized by BALB/c antibodies, but not by CBA/J or CBA/N antibodies. Since CBA/J mice eliminate G. muris, this cannot be a crucial antigen. In addition, he

identified a 40K antigen and a 70K antigen, both of which were recognized by BALB/c, CBA/J and CBA/N antibodies.

The present study extended the previous one by Snider (1985), which was restricted to trophozoite surface antigens. The Western blot technique allowed examination of surface, as well as internal, antigens. The 40K and 70K trophozoite antigens, that were identified by Western blot, may be the surface antigens that were identified by Snider (1985). The Western blot technique additionally identified antigens of 30K, 60K and 65K. The 30K and 80K antigens probably correspond to the 32K and 82K antigens identified by Erlich et al. (1983), using biosynthetic radiolabelling and immunoprecipitation techniques In addition, the Western blot technique identified a 100K G. muris cyst antigen. This may correspond to the 102K G. lamblia cyst antigen described by Reiner, Douglas and Gillin (1989).

The use of the Western blot technique to test the hypothesis that CBA/N mice develop chronic giardiasis because they fail to recognize a crucial G. muris antigen, has certain limitations. A Western blot analysis includes only protein and glycoprotein antigens. Furthermore, I found that the technique was not very sensitive; the best results were obtained at relatively low dilutions of immune (1:25) and hyperimmune (1:100) anti-G. muris antisera. I attempted to analyze, by Western blot, antigen recognition by antibodies present in intestinal lavage fluids obtained from infected mice. This attempt was unsuccessful due to the low concentration of antibodies in intestinal lavage fluids. Thus, the failure of the Western blot technique to identify a specificity defect in CBA/N mice does not rule out the hypothesis that CBA/N mice fail to

recognize a crucial G. muris antigen. The technique may not be sensitive enough to detect antibody recognition of a minor, but crucial, antigen, that is recognized only by mice that eliminate G. muris. Another possibility is that the crucial antigen comigrates with an antigen that is recognized by CBA/N mice; in this case, differential recognition of the antigen by CBA/N and CBA/J or CBA/Ca mice would be concealed. It is also possible that elimination of G. muris may require antibody binding to a particular epitope on an antigen. If CBA/N antibody binds to other epitopes on this antigen, then such a subtle specificity defect would be masked. Finally, the crucial G. muris antigen may not be a protein or glycoprotein antigen. In this case, it would not be detected by the Western blot technique.

Apolar components of G. muris trophozoites were extracted, using organic solvents, for the purpose of examining lipid and glycolipid antigens. Jarroll et al. (1981) previously examined, by thin layer chromatography, lipid components of G. lamblia trophozoites. They identified phosphatidylcholine, phosphatidylethanolamine, sphingomyelin, neutral lipids and 5 glycolipids. However, they did not analyze antibody recognition of these components.

The present study employed a dot blot procedure to compare recognition of G. muris lipid and glycolipid components by sera from CBA/N, CBA/J and BALB/c mice. IgM in immune and normal sera from CBA/J and BALB/c mice bound to a component in the lipid extract (Figures 11 and 12). This component was tentatively identified as a glycolipid, based on its staining by the orcinol-sulphuric acid reagent (Figure 13). This component was one of nine glycolipids visible on the thin layer

chromatogram. It had a relative mobility value (Rf) of 0.45, in the solvent system employed. Thus, this glycolipid had an intermediate polarity. A more polar glycolipid, that remained near the origin on the chromatogram, also bound IgM from CBA/J sera. This spot was more intense on the chromatogram that was exposed to sera from infected CBA/J mice, compared to the chromatogram that was exposed to sera from non-infected CBA/J mice. Thus, this glycolipid may be an antigen for which natural antibody exists in CBA/J mice, and for which more IgM antibody is induced by G. muris infection.

CBA/N sera did not demonstrate the glycolipid-binding activity that was present in CBA/J and BALB/c sera, even when equal amounts of IgM were tested (Figure 12).

Myeloma IgM proteins from BALB/c mice bound to the G. muris glycolipid (Figure 12). One of these (IgMλ (5-8)) is known to be specific for the hapten, dinitrophenol (Azuma et al. 1984). The antigen binding specificity of the other (IgMκ (HPC76)) is not known (Bernhard and Gough 1980). Binding of these myeloma IgM proteins to the G. muris glycolipid suggests that the binding reactions observed are non-immunologic. That is, that the binding of the G. muris glycolipid by CBA/J and BALB/c, immune and normal, sera probably does not represent antibody activity. Consequently, the differential binding of the G. muris glycolipid by CBA/N IgM, and CBA/J and BALB/c IgM, probably does not represent a difference in the ability of these strains to mount an immune response against G. muris. Thus, the analysis of G. muris lipid and glycolipid components also failed to reveal a specificity defect in the antibody response of

CBA/N mice. As such, this analysis did not contribute to our understanding of immunity to G. muris.

Nevertheless, these results are interesting because they point out a possible difference in the structure of IgM from CBA/N mice, and IgM from CBA/J and BALB/c mice. The differential binding of the G. muris glycolipid by CBA/N IgM, and CBA/J and BALB/c IgM, was not due to a quantitative difference in the levels of serum IgM between these strains (Figure 12). Therefore, there must be a qualitative difference between CBA/N IgM and CBA/J and BALB/c IgM. Since the Xid gene is located outside the Igh locus, this difference cannot be in the primary structure of the mu chain. However, there may be a difference in the post-translational modification of IgM. One possibility is that the normal allele of the Xid gene encodes a glycosyl transferase, or a product is involved in the regulation of the glycosylation process. The Xid mutation may alter or prevent the expression of this activity in CBA/N mice, thus resulting in a structural defect in IgM in this strain. The possibility of a structural defect in IgM has profound implications for the molecular basis of the Xid phenotype, including the defective maturation of B lymphocytes. Thus, although these observations did not contribute to our understanding of the immune mechanisms involved in the elimination of G. muris, further study in this area may contribute to our understanding of the biology of the CBA/N mouse and the role of the X-chromosome in the development and regulation of the immune system.

3.4 Resistance of CBA/N Mice to Reinfection with G. muris

The mechanisms of immunity that mediate elimination of a primary infection are not necessarily the same as those that mediate protection against reinfection with the same infectious agent (Mitchell, McMichael and Lamb 1985). The purpose of these experiments was to determine whether CBA/N mice, which fail to eliminate a primary infection with G. muris, are able to resist a secondary challenge infection with G. muris, following drug-cure of their primary infection.

Immunocompetent strains of mice, that eliminate a primary infection with G. muris, are resistant to a subsequent challenge infection with G. muris (Roberts-Thomson et al. 1976; Brett and Cox 1982). Belosevic and Faubert (1983b) studied the time course of acquired resistance to giardiasis in several strains of mice. They found that mice whose primary infections were terminated after 3 weeks by treatment with metronidazole were partially protected against reinfection with G. muris. That is, their cyst output was lower than that of mice undergoing a primary infection. Mice whose primary infections were terminated after 7 weeks were completely protected against reinfection with G. muris. That is, these mice had undetectable cyst output in their feces. These results are similar to previous results obtained by Underdown et al. (1981). This group of investigators additionally found, quite surprisingly, that C3H/He

mice, which failed to eliminate a primary infection with G. muris, were able to resist reinfection with G. muris.

Thus, I considered the hypothesis that, if the immune mechanisms that mediate elimination of G. muris are different from those that mediate resistance to reinfection with G. muris, then CBA/N mice may be able to resist a secondary challenge infection with G. muris, provided that the latter mechanisms are intact in CBA/N mice.

3.4.1 Results

3.4.1.1 Resistance of CBA/N and CBA/Ca Mice to Reinfection with G. muris After Drug-cure of a Primary Infection

CBA/N and CBA/Ca mice were infected with G. muris at various time points. These primary infections were terminated, simultaneously, by treatment with metronidazole. The durations of these primary infections were 3, 6 and 9 weeks. Seven days after termination of the primary infections, the mice were challenged by reinfection with G. muris cysts. The levels of G. muris cyst output by the challenged mice were compared to those of mice undergoing a primary infection. These results are shown in Table 7. Both CBA/N and CBA/Ca mice, whose primary infections were of 6 or 9 weeks' duration, were completely resistant to the secondary challenge infection. These mice had undetectable cyst output in their feces at week one of the challenge infection. Protection was confirmed by undetectable cyst output at weeks 2 and 3 of the challenge infection (data not shown).

CBA/Ca mice, whose primary infections had been of 3 weeks' duration, were partially protected against the secondary challenge infection. Cyst output by these mice, at week one of the challenge infection, was much lower than that of CBA/Ca mice undergoing a primary infection. Moreover, these mice had almost eliminated the parasite by

TABLE 7

RESISTANCE OF CBA/N MICE TO A SECONDARY CHALLENGE INFECTION WITH *C. MURIS*: DEPENDENCE ON DURATION OF PRIMARY INFECTION

DURATION OF PRIMARY INFECTION[a]	MICE	LEVEL OF SECONDARY INFECTION IN CHALLENGED MICE (CYSTS PER 8 FECAL PELLETS)[b]		
		WEEK 1	WEEK 5	WEEK 7
0	CBA/N	320,175 (282,100-363,470)[c]	74,296 (53,991-103,554)[c]	24,708 (21,438-28,481)[c]
	CBA/Ca	262,802 (243,923-287,470)	13,296 (9,646-18,329)	5,385 (4,124-7,033)
3	CBA/N	2,650 (1,904-3,689)	4,304 (2,407-7,696)	8,259[d] (5,043-13,524)
	CBA/Ca	2,785 (2,073-3,741)	<550 (-)	884[e] (724-983)
6	CBA/N	<550 (-)	N/D	N/D
	CBA/Ca	<550 (-)	N/D	N/D
9	CBA/N	<550 (-)	N/D	N/D
	CBA/Ca	<550 (-)	N/D	N/D

a The primary infection was terminated by treatment with metronidazole (see 2.4); the secondary challenge infection was given one week later.

b Geometric mean of 5 mice.

c Range of SEM.

d 5 of 5 mice infected.

e 1 of 5 mice infected.

N/D not done.

week 5 of the challenge infection. One of five of these mice had a small cyst output at week 7.

CBA/N mice, whose primary infections had been of 3 weeks' duration, were partially protected against the secondary challenge infection. Cyst output by these mice at week one of the challenge infection, was much lower than that of CBA/N mice undergoing a primary infection. However, cyst output by the challenged, CBA/N mice did not quickly decrease thereafter, as did cyst output by the challenged, CBA/Ca mice. Rather, their cyst output was greater at weeks five and seven of the challenge infection. This experiment was repeated, and cyst output by the challenged, CBA/N mice and by CBA/N mice undergoing a primary infection, were closely followed at weekly intervals. These results are shown in Figure 14. The previously observed pattern of infection was confirmed. That is, CBA/N mice whose primary infections had been of 3 weeks' duration, were both partially protected against, and susceptible to, a secondary challenge infection with G. muris. The levels of cyst output by these mice were low in the early weeks of the challenge infection. In later weeks, however, their cyst output increased to the chronic levels of cyst output by CBA/N mice undergoing a primary infection. The secondary challenge infections in CBA/N mice, whose primary infections were of 3 weeks' duration, were chronic. That is, these mice failed to eliminate the secondary challenge infection with G. muris.

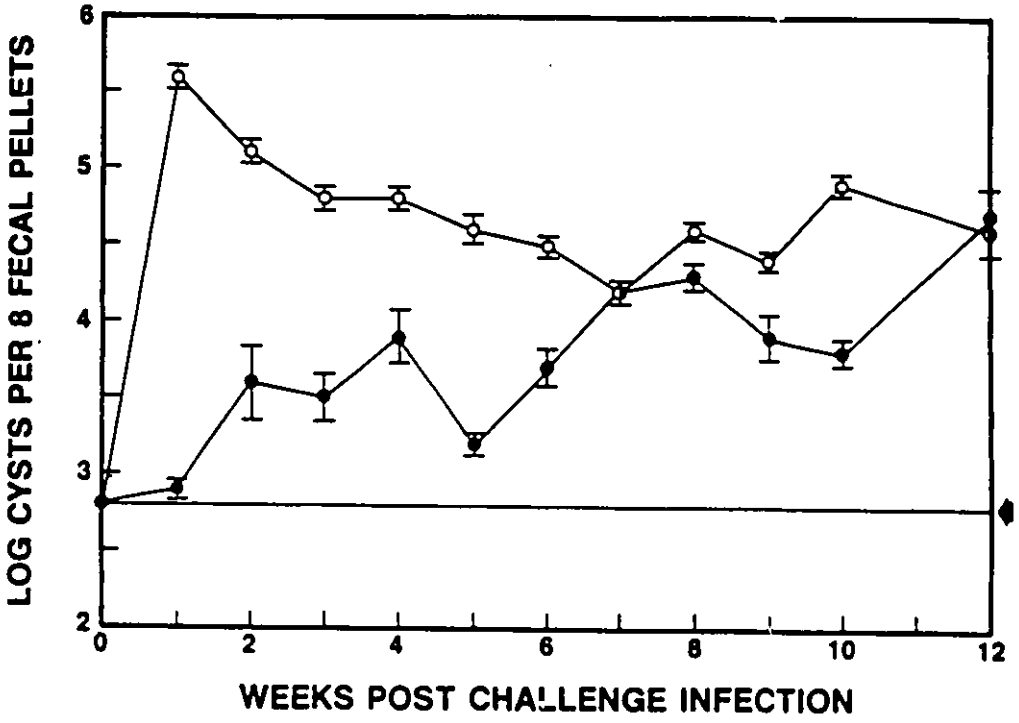
3.4.1.2 Reconstitution of Irradiated CBA/N and CBA/Ca Mice with Spleen Cells from G. muris-immune CBA/N CBA/Ca Mice

Since CBA/N and CBA/Ca mice, whose primary infections were of 6 or 9 weeks' duration, were immune to subsequent G. muris infection, I attempted to transfer this resistance to lethally irradiated recipients. Spleen cells from the G. muris-immune, CBA/N and CBA/Ca mice were injected into lethally irradiated, CBA/N or CBA/Ca mice. Three weeks later, the reconstituted mice were infected with G. muris. The levels of cyst output by these mice were compared to the levels of cyst output by intact CBA/N and CBA/Ca mice undergoing primary G. muris infections. These results are shown in Figure 15. The lethally irradiated recipients of spleen cells from G. muris-immune mice, were not immune to G. muris infection. The peak of mean cyst output by the reconstituted mice was equivalent to the peak of mean cyst output by mice undergoing primary G. muris infections. However, the lethally irradiated recipients of spleen cells from G. muris-immune, CBA/Ca mice, eliminated the parasite much more quickly than did intact CBA/Ca mice that had a primary G. muris infection. The former mice had eliminated G. muris by week four of the challenge infection, while the latter mice eliminated G. muris after week 10. The course of G. muris infection in the lethally irradiated recipients of spleen cells from G. muris-immune, CBA/N mice, was similar to the course of a primary G. muris infection in intact CBA/N mice. That is, lethally irradiated mice, that were reconstituted with spleen cells from G. muris-immune CBA/N mice, developed chronic giardiasis. The results of this adoptive transfer

Figure 14

CBA/N mice, that had been infected with G. muris for 3 weeks (●), were cured of their infections by treatment with metronidazole. Control CBA/N mice (○), that had not been infected (0 weeks), were also treated with the drug. A secondary challenge infection was given (at week 0) by peroral administration of 5000 G. muris cysts. The course of the challenge infection was followed by measuring cyst output in feces. Each point represents the mean of values from 5 mice. Error bars represent the standard error of the mean. The arrow marks the limit of detection (600 cysts per 8 fecal pellets).

SUSCEPTIBILITY OF CBA/N MICE TO A SECONDARY CHALLENGE INFECTION WITH G. MURIS FOLLOWING A PRIMARY INFECTION OF THREE WEEK DURATION

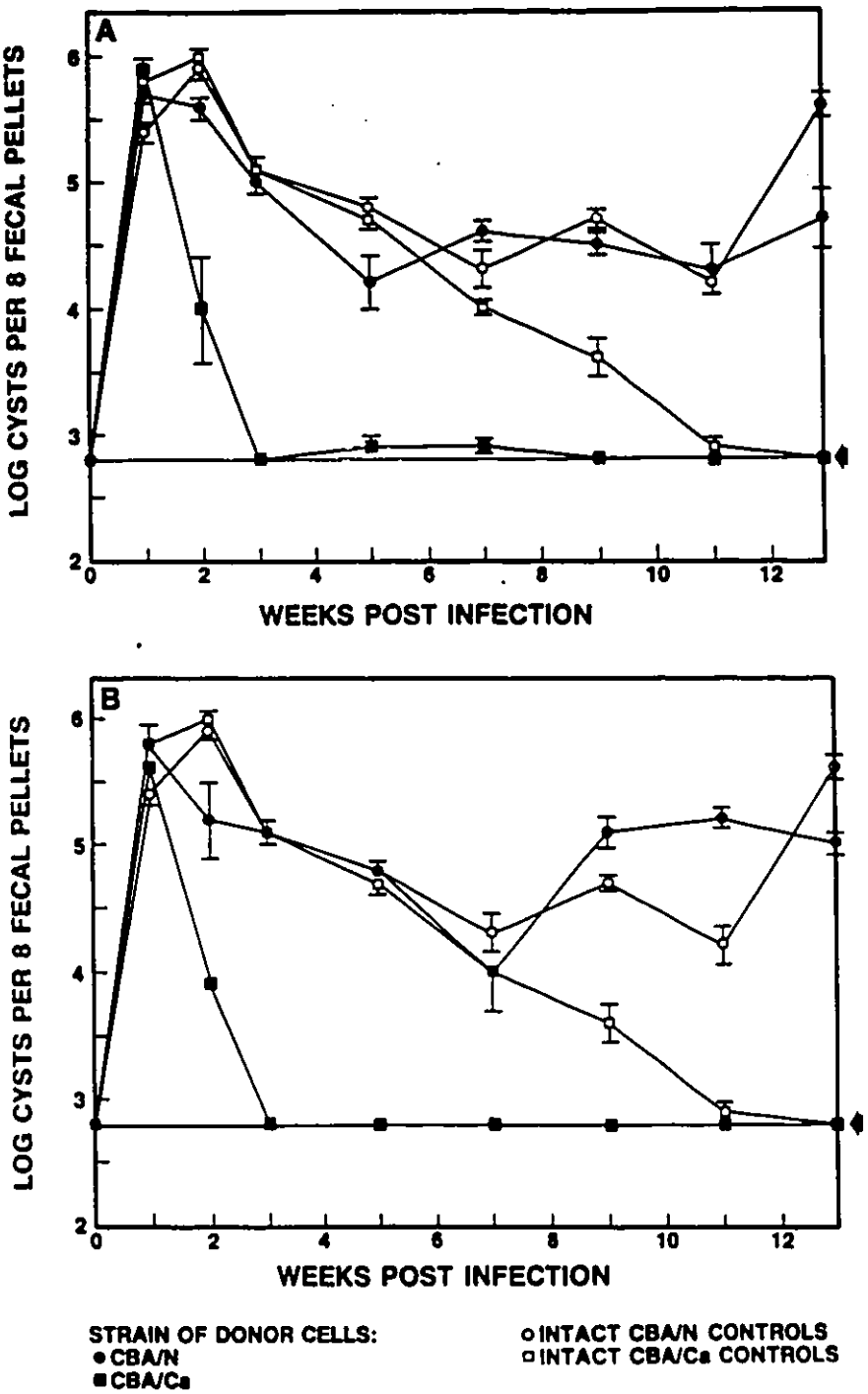


LENGTH OF PRIMARY INFECTION:
● 3 WEEKS
○ 0 WEEKS

Figure 15

(A) CBA/N mice and (B) CBA/Ca mice were lethally irradiated by exposure to 1000 rads from a ^{137}Cs source. Immediately following the irradiation, the mice were reconstituted with $50X10^6$ splenocytes from either CBA/N (●) or CBA/Ca (■) mice. The splenocyte-donor mice had previously been infected with G. muris for more than 6 weeks; they had been cured of their primary infections by treatment with metronidazole, and were completely resistant to a secondary challenge infection, that is, they were G. muris-immune. Three weeks after the reconstitution (at week 0), the reconstituted mice, together with intact (not previously infected) CBA/N mice (○) and intact (not previously infected) CBA/Ca mice (□), were infected by peroral administration of 5000 G. muris cysts. The course of infection was followed by measuring cyst output in feces. Each point represents the mean of values from 4-5 mice. Error bars represent the standard error of the mean. The arrow marks the limit of detection (600 cysts per 8 fecal pellets).

ADOPTIVE TRANSFER OF SPLEEN CELLS FROM G. MURIS-IMMUNE CBA/N AND CBA/Ca MICE TO IRRADIATED CBA/N (A) AND CBA/Ca (B) MICE: COURSE OF G. MURIS INFECTION IN RECONSTITUTED MICE



experiment were the same, whether CBA/N mice or CBA/Ca mice served as the lethally irradiated recipients, and were reproduced in one other separate experiment.

3.4.2 Discussion

CBA/N mice fail to eliminate a primary infection with G. muris. However, if the primary infection is terminated, by treatment with metronidazole, then CBA/N mice become resistant to reinfection with G. muris (Table 7). This acquired resistance of CBA/N mice occurs in the same time frame as the acquired resistance of other strains of mice (Underdown et al. 1981; Belosevic and Faubert 1983b). These results suggest that the immune mechanisms that mediate elimination of a primary G. muris infection are different from those that mediate resistance to reinfection with G. muris. The former mechanisms are defective in CBA/N mice, while the latter mechanisms are intact. Both mechanisms are intact in CBA/Ca mice.

The remainder of the results presented in this section can be explained in terms of a model. The model holds that there are two mechanisms, or sets of mechanisms, that mediate immunity to G. muris. The first mechanism, or set of mechanisms, that mediates elimination of a primary G. muris infection, is active against large numbers of trophozoites that are already established, that is, attached to the epithelial surface. The second mechanism, or set of mechanisms, that mediates resistance to reinfection, is active against smaller numbers of trophozoites, immediately after excystation, that is, before the

trophozoites are attached to the epithelium. The first mechanism is intact in CBA/Ca mice, but is defective in CBA/N mice. The second mechanism is intact in both strains of mice.

Both mechanisms, or sets of mechanisms, are induced by a primary infection with G. muris. Full stimulation of the second mechanism, that mediates resistance to reinfection, requires a primary infection of greater than 3 weeks' duration. Therefore, mice whose primary infections were of 6 or 9 weeks' duration, were completely protected against a secondary challenge infection (Table 7). However, mice whose primary infections were of only 3 weeks' duration, were only partially protected against the challenge. In these mice, there was some resistance to excystation-derived trophozoites. However, some of the excystation-derived trophozoites were able to escape the partially stimulated second mechanism of immunity, and colonize the epithelium of the small intestine.

CBA/Ca mice are capable of eliminating established trophozoites from the intestinal epithelium. In this experiment, the CBA/Ca mice eliminated the secondary challenge infection, very quickly, because the first mechanism of immunity to G. muris was stimulated by the primary infection. However, this first mechanism of immunity to G. muris, that mediates elimination of established trophozoites, is defective in CBA/N mice. Therefore, in these mice, the excystation-derived trophozoites, that escaped the partially-stimulated second mechanism of immunity, colonized the small intestinal epithelium, and failed to be eliminated. Thus, the CBA/N mice, whose primary infections were of 3 weeks' duration, developed a low level of infection upon challenge, that increased and became chronic (Figure 14).

Due to the CBA/N's defect in the first mechanism of immunity to G. muris (that mediates elimination of established trophozoites), if the challenge infection is not completely terminated at the point of excystation (by the second mechanism of immunity of G. muris, that mediates resistance to reinfection), then the challenge infection becomes a chronic infection.

The results of the adoptive transfer can also be explained in terms of this model. The adoptive transfer of spleen cells from G. muris-immune CBA/N and CBA/Ca mice, to lethally irradiated recipient mice, failed to transfer resistance to G. muris (Figure 15). These results indicate that the second mechanism of immunity to G. muris, that mediates resistance to reinfection with the parasite, is not present in the spleen. Since G. muris is an intestinal parasite, this result is not surprising.

Underdown et al. (1988) have shown that pre-incubation of G. muris trophozoites with intestinal lavage fluids containing secretory IgA antibody derived from G. muris-infected BALB/c mice, prevents the establishment of G. muris infection when these trophozoites are transferred, by laparotomy, to the intestines of naive BALB/c mice. Preliminary results from this laboratory showed that intestinal secretions from G. muris-infected CBA/N and CBA/Ca mice were equally effective at preventing G. muris infection, in this system (B. Underdown, personal communication). These results support the hypothesis that secretory IgA antibody provides the second mechanism of immunity, that mediates resistance to reinfection with G. muris. This hypothesis is consistent with our observations that CBA/N mice make quantitatively normal levels

of secretory IgA antibody in response to infection with G. muris (Snider, Skea and Underdown 1988).

Although resistance to G. muris infection was not transferred to lethally irradiated recipients by spleen cells from G. muris-immune CBA/N and CBA/Ca mice, the CBA/Ca cells did transfer the ability to eliminate the parasite more quickly (Figure 15). I previously showed that spleen cells from non-infected, CBA/Ca mice transferred, to lethally irradiated recipients, the ability to eliminate a primary G. muris infection (Figure 3). Thus, these results indicate that the first mechanism of immunity to G. muris, that mediates elimination of established trophozoites, is inducible by G. muris infection, and is present, in the form of cells, in the spleen. Spleen cells from G. muris-immune CBA/N mice did not transfer any form of resistance of G. muris; the course of G. muris infection in lethally irradiated recipients of these cells was indistinguishable from the course of a primary G. muris infection in intact CBA/N mice (Figure 15). This result was expected, since the first mechanism of immunity to G. muris, that mediates elimination of established trophozoites, is defective in CBA/N mice.

In summary, there appear to be two mechanisms, or sets of mechanisms, that mediate immunity to G. muris. The first of these mediates elimination of trophozoites that are established on the epithelium of the small intestine. This mechanism is defective in CBA/N mice, but is intact in CBA/Ca mice. It is inducible by G. muris infection, and is transferrable with spleen cells The second mechanism mediates resistance to the establishment of G. muris infection. It must be present at the time of introduction of trophozoites into the small intestine. It is intact in

both CBA/N and CBA/Ca mice, and is not transferable with spleen cells. This mechanism appears to be intestinal, secretory IgA antibody.

Chapter 4

CONCLUSIONS

The objective of the work described in this thesis was to contribute to our understanding of the mucosal immune system by attempting to elucidate the mechanisms of immunity that contribute to host resistance against an intestinal parasite. The CBA/N mouse model of B cell deficiency was employed to study the requirement for B cells and antibody in the elimination of the protozoan parasite, G. muris.

Unlike most immunocompetent strains of mice, CBA/N mice fail to eliminate G. muris. The results of the cellular reconstitution experiments (section 3.1) showed that lethal irradiation of CBA/N mice, followed by reconstitution with spleen cells from the immunocompetent strain, CBA/Ca, provided the ability to eliminate G. muris. Reconstitution of lethally irradiated mice with spleen cells from CBA/N mice resulted in susceptibility to chronic giardiasis. These results indicate that chronic giardiasis in CBA/N mice is due to an immune defect, and that CBA/Ca spleen contains cells that are crucial for the elimination of G. muris.

The failure of CBA/Ca spleen cells to reconstitute the ability to eliminate G. muris in non-irradiated, CBA/N mice indicates that the

provision of immunocompetent, mature B cells is insufficient to allow elimination of G. muris. Moreover, the failure of separated, CBA/Ca B cells and T cells to reconstitute the ability to eliminate G. muris in lethally irradiated, CBA/N recipients, indicates that conventional B cells and T cells, and their precursors, are insufficient for elimination of G. muris. These results do not rule out the importance of conventional B cells and T cells in the elimination of G. muris. However, they suggest that, in addition, another non-conventional cell type is required.

This cell type was depleted by procedures designed to selectively deplete B cells and T cells, and so, may be present in the spleen at a very low frequency. A possible candidate for this cell type is the Ly1+ B cell. Ly1+ B cells are absent in CBA/N mice (Hayakawa, Hardy and Herzenberg 1986), they are present at low frequency in the spleens of normal, adult mice (Herzenberg et al. 1986), they non-specifically stick to plastic surfaces (L. Herzenberg, personal communication) and they contribute to intestinal B cell populations (Kroese et al. 1989). Moreover, Ly1+ B cells, like conventional B cells, are depleted by treatment of mice from birth with anti-IgM antibody (Herzenberg et al. 1986). Anti-IgM antibody-treated mice also fail to eliminate G. muris (Snider et al. 1985). Thus, the absence of Ly1+ B cells in CBA/N mice may be the cause of their susceptibility to chronic giardiasis. This hypothesis was tested by reconstituting lethally irradiated mice with CBA/N spleen cells (which do not provide the ability to eliminate G. muris), supplemented with CBA/Ca peritoneal cells (which contain a relatively large proportion of Ly1+ B cells (Herzenberg et al. 1986)). At the time of writing, this experiment was in progress.

If Lyl+ B cells are crucial for the elimination of G. muris, then what is their contribution to this process? One possibility is that Lyl+ B cells produce antibodies of the crucial specificities required for elimination of the parasite. However, I failed to detect a specificity defect in the antibody response of CBA/N mice to G. muris (section 3.3). It is known that Lyl+ B cells are the major producers of autoantibodies, including anti-idiotypic antibodies, in normal, adult mice (reviewed in Herzenberg et al. 1986). Thus, another possibility is that Lyl+ B cells produce antibodies that react with the idiotypes of anti-G. muris antibodies, and that an intact idiotype-anti-idiotype network is crucial for the elimination of G. muris. For example, if parasite-protective antibodies are produced, then antibodies directed at the idiotype of these antibodies would block their binding to the parasite, thus preventing their protective effect. This inhibition would then allow host-protective antibodies to bind to the parasite and mediate its elimination. Alternatively, anti-idiotypic antibody may combine with the antibody-parasite complex, and this additional antibody may be the final trigger for parasite elimination. Another possibility is that anti-idiotypic antibodies may be involved in the regulation of production of host-protective versus parasite-protective antibodies. Such a subtle defect in the anti-idiotype response of CBA/N mice may contribute to their susceptibility to chronic giardiasis.

In summary, cellular reconstitution experiments involving CBA/N mice failed to identify the cell type, that is missing or defective in these mice, that is crucial for elimination of G. muris. It was shown that this cell type is present in CBA/Ca spleen. However, it did not

appear to be a conventional B cell or T cell. Conventional B cells and T cells may be important in the elimination of G. muris, however, they are insufficient. There appears to be another, rather unusual, cell type that is necessary for the elimination of G. muris. Thus, the defect of CBA/N mice that renders them susceptible to chronic giardiasis is more complicated than just the defect in conventional B cell maturation. The defect in conventional B cells in CBA/N mice may contribute to their susceptibility to chronic giardiasis. However, there must be an additional reason for the failure of CBA/N mice to eliminate G. muris. Lyl+ B cells, which are absent in CBA/N mice, may contribute to elimination of the parasite, or there may be another, non-B, non-T cell type, that has not yet been identified, that is crucial for elimination of G. muris.

It was previously suggested that serum IgG antibody may be important for the elimination of G. muris (Snider 1985). This hypothesis was based on the observation that CBA/N mice made a quantitatively deficient serum IgG antibody response to infection with G. muris (Snider, Skea and Underdown 1988). IgG anti-G. muris antibody was not detected in the intestinal secretions of G. muris-infected, immunocompetent mice (Snider and Underdown 1986). Nonetheless, it was suggested that serum IgG antibody may neutralize a G. muris secretory product that is essential for parasite survival in the intestine (Snider 1985). Alternatively, serum IgG anti-G. muris antibody may cooperate with cells, which, in combination, migrate into the intestine to attack the parasite (Snider 1985). The results presented in this thesis (section 3.2) showed that the provision of serum IgG anti-G. muris antibody to CBA/N mice failed to

induce elimination of G. muris. Thus, these results do not support the hypothesis suggested by Snider (1985). The results do not strictly rule out a role for IgG antibody in the elimination of G. muris. However, they indicate that the deficient serum IgG antibody response of CBA/N mice is not solely responsible for their susceptibility to chronic giardiasis.

The analysis of antigen recognition by antibodies from CBA/N mice, compared to antigen recognition by antibodies from immunocompetent mice, failed to reveal a specificity defect in the anti-G. muris antibody response of CBA/N mice (section 3.3). These results do not rule out the hypothesis that CBA/N mice fail to eliminate G. muris because they fail to recognize a crucial antigen on the parasite. However, they do indicate that, if such a specificity defect does exist, then it is not an obvious one. The techniques used may not have been sensitive enough to detect differential recognition of a minor, but crucial, antigen. Alternatively, the techniques used may have been inappropriate, if the specificity defect is something other than antibody recognition of a protein- or lipid-based antigen. One possibility is that the specificity defect is not the absence of antibody directed at a particular antigen, but rather the absence of antibody directed at the idiotype of a specific anti-G. muris antibody. The anti-idiotype antibody response of mice to G. muris infection has never been analyzed.

One interesting and serendipitous finding derived from the antigen recognition studies was the indication of a possible structural defect in IgM from CBA/N mice. IgM is an important surface molecule, expressed early in the differentiation of B lymphocytes (Huber 1982). Thus, a defect in the structure of IgM may have profound effects on the

maturation of B cells. The elucidation of the nature of the structural defect in CBA/N IgM may contribute to our understanding of the molecular basis of the Xid phenotype.

Finally, the studies of giardiasis in CBA/N mice indicated that the mechanisms of immunity that mediate elimination of a primary G. muris infection are different from those that mediate resistance to reinfection (section 3.4). While the former mechanisms are defective in CBA/N mice, the latter are intact.

At the present time, we lack a complete understanding of the mechanisms that mediate elimination of a primary infection with G. muris. Certainly, helper T cells and B cells are crucial for elimination of G. muris (Heyworth, Carlson and Ermak 1987; Snider et al. 1985). However, T cells and B cells were insufficient to reconstitute the ability to eliminate G. muris, in lethally irradiated recipients. Thus, another cell type appears to be required for elimination of this parasite. This cell type is present, probably at a low frequency, in CBA/Ca spleen. The G. muris antigens that stimulate immune elimination of the parasite have not yet been identified. I failed to detect a specificity defect in the serum antibody response of CBA/N mice to G. muris infection. Based on all of these observations, I propose the hypothesis that Lyl+ B cells contribute to the elimination of G. muris by producing antibodies that react with the idiotypes of the anti-G. muris antibodies that are produced by conventional B cells. Studies of this hypothesis would advance our knowledge of the contribution of Lyl+ B cells and idiotype-anti-idiotype networks in mucosal immunity.

The mechanisms of immunity that mediate resistance to reinfection

with G. muris may be less obscure than those that mediate elimination of a primary infection. These mechanisms are intact in CBA/N mice. CBA/N mice make quantitatively normal levels of intestinal IgA antibody in response to G. muris infection (Snider and Underdown 1986). Moreover, resistance to G. muris infection can be passively transferred, with intestinal IgA, anti-G. muris antibody, to the intestines of naive mice. Thus, the available evidence indicates that secretory IgA antibody mediates resistance to reinfection with G. muris.

REFERENCES

Ahmed, A., I. Scher, S.O. Sharrow, A.H. Smith, W.E. Paul, D.H. Sachs and K.W. Sell. 1977. B-lymphocyte heterogeneity: Development and characterization of an alloantiserum which distinguishes B-lymphocyte differentiation alloantigens. J. Exp. Med. 145:101-10.

Amsbaugh, D.F., C.T. Hansen, B. Prescott, P.W. Stashak, D.R. Barthold and P.J. Baker. 1972. Genetic control of the antibody response to type III pneumococcal polysaccharide. I. Evidence that an X-linked gene plays a decisive role in determining responsiveness. J. Exp. Med. 136:931-49.

Anders, R.F., I.C. Roberts-Thomson and G.F. Mitchell. 1982. Giardiasis in mice: Analysis of humoral and cellular immune responses to Giardia muris. Parasite Immunol. 4:47-57.

Andrews, J.S., and E.L. Hewlett. 1981. Protection against infection with Giardia muris by milk containing antibody to Giardia. J. Inf. Dis. 143:242-6.

Azuma, T., V. Igras, E.B. Reilly and H.N. Eisen. 1984. Diversity at the variable-joining region boundary of lambda light chains has a pronounced effect on immunoglobulin ligand-binding activity. Proc. Natl. Acad. Sci. (USA) 81:6139-43.

Belosevic, M., and G.M. Faubert. 1983a. Giardia muris: Correlation between oral dosage, course of infection and trophozoite distribution in the mouse small intestine. Exp. Parasitol. 56:93-100.

Belosevic, M., and G.M. Faubert. 1983b. Temporal study of acquired resistance in infections of mice with Giardia muris. Parasitol. 87:517-24.

Belosevic, M., G.M. Faubert, E. Skamene and J.D. MacLean. 1984. Susceptibility and resistance of inbred mice to Giardia muris. Infect. Immun. 44:282-6.

Belosevic, M., and G.M. Faubert. 1986a. Killing of Giardia muris trophozoites in vitro by spleen, mesenteric lymph node and peritoneal cells from susceptible and resistant mice. Immunol. 59:269-75.

Belosevic, M., and G.M. Faubert. 1986b. Comparative studies of inflammatory responses in susceptible and resistant mice infected with Giardia muris. Clin. Exp. Immunol. 65:622-30.

Belosevic, M., and G.M. Faubert. 1987. Lysis and immobilization of Giardia muris in vitro by immune serum from susceptible and resistant mice. Parasite Immunol. 9:11-9.

Bernhard, O., and N.M. Gough. 1980. Nucleotide sequence of immunoglobulin heavy chain joining segments between translocated V_H and mu constant region genes. Proc. Natl. Acad. Sci. (USA). 77:3630-4.

Bienenstock, J., and A.D. Befus. 1980. Mucosal immunology. Immunol. 41:249-70.

Bingham, A.K., and E.A. Meyer. 1979. Giardia excystation can be induced in vitro in acidic solutions. Nature 277:301-2.

Black, R.E, A.C. Dykes, S.P. Sinclair and J.G. Wells. 1977. Giardiasis in day-care centres: Evidence of person-to-person transmission. Pediatrics 60:486-91.

Boorman, G.A., P.H.C. Lina, C. Zurcher and H.T.M Nieuwerkerk. 1973. Hexamita and giardia as a cause of mortality in congenitally thymus-less (nude) mice. Clin. Exp. Immunol. 15:623-7.

Boswell, H.S., M.I. Nerenberg, I. Scher and A. Singer. 1980. Role of accessory cells in B cell activation. III. Cellular analysis of primary immune response deficits in CBA/N mice: Presence of an accessory cell - B cell interaction defect. J. Exp. Med. 152:1194-209.

Bradley, L.M. 1980. Cell proliferation. In Selected Methods in Cellular Immunology, ed. B.B. Mishell and M.M. Shiigi, 153-72. San Francisco: W.H. Freeman and Co.

Brett, S.J., and F.E.G. Cox. 1982. Immunological aspects of Giardia muris and Spironucleus muris infections in inbred and outbred strains of laboratory mice: A comparative study. Parasitol. 85:85-99.

Brinkmann, V., J.S. Remington and S.D. Sharma. 1987. Protective immunity in Toxoplasmosis: Correlation between antibody response, brain cyst formation, T-cell activation and survival in normal and B-cell deficient mice bearing the H-2^k haplotype. Infect. Immun. 55:990-4.

Brockhaus, M., J.L. Magnanni, M. Blaszczyk, Z. Steplewski, H. Koprowski, K.A. Karlsson, G. Larson and V. Ginsburg. 1981. Monoclonal antibodies directed against the human Le^b blood group antigen. J. Biol. Chem. 256:13,223-5.

Butscher, W.G., G.M. Faubert. 1988. The therapeutic action of monoclonal antibodies against a surface glycoprotein of Giardia muris. Immunol. 64:175-80.

Carlson, J.R., M.F. Heyworth and R.L. Owen. 1986a. Response of Peyer's patch lymphocyte subsets to Giardia muris infection in BALB/c mice. I. T-cell subsets. Cell. Immunol. 97:44-50.

Carlson, J.R., M.F. Heyworth and R.L. Owen. 1986b. Response of Peyer's patch subsets to Giardia muris infection in BALB/c mice. II. B-cell subsets: Enteric antigen exposure is associated with immunoglobulin isotype switching by Peyer's patch B-cells. Cell. Immunol. 97:51-8.

Cheng, T.C. 1986. Introduction to the parasitic protozoa. In General Parasitology, 97-151. Orlando: Academic Press.

Christie, W.W. 1982. Lipid Analysis, 2d ed., 34. London: Pergamon Press.

Clark, J.T., and D.V. Holberton. 1986. Plasma membrane isolated from Giardia lamblia: Identification of membrane proteins. Eur. J. Cell Biol. 42:200-6.

Clayberger, C., R.H. DeKruyff, R. Fay, B. Huber and H. Cantor. 1985. Evidence for defects in accessory and T cell subsets in mice expressing the Xid defect. J. Mol. Cell. Immunol. 2:61-9.

Colten, H.R. 1974. Synthesis and metabolism of complement proteins. Transpl. Proc. 6:33-8.

Correa-Oliveira, R., and A. Sher. 1985. Defective immunoglobulin M responses to vaccination or infection with Schistosoma mansoni in Xid mice. Infect. Immun. 50:409-14.

Costello, P.B., and F.A. Green. 1986. Reactivity patterns of human anti-cardiolipin and other anti-phospholipid antibodies in syphilitic sera. Infect. Immun. 51:771-5.

Cuatrecasas, P., and C.B. Anfinson. 1971. Affinity chromatography. Ann. Rev. Immunol. 40: 259-80.

Cunliffe-Beamer, T.L. 1983. Biomethodology and surgical techniques. In The Mouse in Biomedical Research. Volume III Normative Biology. Immunology and Husbandry, ed. H.L. Foster, J.D. Small and J.G. Fox, 401-37. Orlando:Academic Press.

Davis, S.E., M.L. Jaffe, P.J. Munson and D. Rodbard. 1980. RIA data processing with a small programmable calculator. J. Immunoassay 1:15-20.

Deguchi, M., F.D. Gillin and I Gigli. 1987. Mechanism of killing of Giardia lamblia trophozoites by complement. J. Clin. Invest. 79:1296-302.

De la Hera, A., M.A.R. Marcos, M.L. Toribio, C. Marquez and M.L. Gaspar. 1987. Development of Ly-1 B cells in immunodeficient CBA/N mice. J. Exp. Med. 166:804-9.

Den Hollander, N. D. Riley and D. Befus. 1988. Immunology of giardiasis. Parasitol. Today 4:124-31.

Dupont, H.L., and P.S. Sullivan. 1986. Giardiasis: the clinical spectrum, diagnosis and therapy. Ped. Inf. Dis. 5:S131-8.

Duran, L.W., and E.S. Metcalf. 1987. Antibody-defective genetically susceptible CBA/N mice have an altered Salmonella typhimurium-specific B cell repertoire. J. Exp. Med. 165:29-46.

Edson, C.M., M.J.G. Farthing, D.A. Thorley-Lawson and G.T. Keusch. 1986. An 88,000 Mr Giardia lamblia surface protein which is immunogenic in humans. Infect. Immun. 54:621-5.

Einfield, D.A., and H.H. Stibbs. 1984. Identification and characterization of a major surface antigen of Giardia lamblia. Infect. Immun. 46:377-83.

Eldridge, J.H., H. Kiyono, S.M. Michalek and J.R. McGhee. 1983. Evidence for a mature B cell subpopulation in Peyer's patches of young, adult Xid mice. J. Exp. Med. 157:789-94.

Eldridge, J.H., L.J. Yaffe, J.J. Ryan, H. Kiyono, I. Scher and J.R. McGhee. 1984. Expression of Lyb-5 and Mls determinants by Peyer's patch B lymphocytes of X-linked immunodeficient mice. J. Immunol. 133:2308-11.

Eldridge, J.H., K. W. Beagley and J.R. McGhee. 1987. Immunoregulation in the Peyer's patch microenvironment. Cellular basis for the enhanced responses by the B cells of X-linked immunodeficient CBA/N mice. J. Immunol. 139:2255-62.

Eldridge, J.H., J.A. Meulbroek and J.R. McGhee. 1988. Elevated total serum IgA and supranormal IgA responses to Type I and T-cell dependent antigens in X-linked immunodeficient mice. (abstract) The F.A.S.E.B. J. 2:A893.

Erlandsen, S.L., and D.E. Feely. 1984. Trophozoite motility and the mechanism of attachment. In Giardia and Giardiasis, ed. S.L. Erlandsen and E.A. Meyer, 33-64. New York: Plenum Press.

Erlich, J.H., R.F. Anders, I.C. Roberts-Thomson, J.W. Schrader and G.F. Mitchell. 1983. An examination of differences in serum antibody specificities and hypersensitivity reactions as contributing factors to chronic infection with the intestinal protozoan parasite, Giardia muris, in mice. Aust. J. Exp. Biol. Med. Sci. 61:599-615.

Ernst, P.B., A.D. Befus and J. Bienenstock. 1985. Leukocytes in the intestinal epithelium: an unusual immunological compartment. Immunol. Today 6:50-5.

Farthing, M.J.G., M.E.A.Pereira and G.T. Keusch. 1986. Description and characterization of a surface lectin from Giardia lamblia. Infect. Immun. 51:661-7.

Feely, D.E., S.L. Erlandsen and D.G. Chase. 1984. Structure of the trophozoite and cyst. In Giardia and Giardiasis, ed. S.L. Erlandsen and E.A. Meyer, 3-32. New York: Plenum Press.

Fidler, J.M., E.L. Morgan and W.O. Weigle. 1980. B lymphocyte differentiation in the CBA/N mouse: A delay in maturation rather than a total arrest. J. Immunol. 124:13-19.

Gillin, F.D., D.S. Reiner, M.J. Gault, H. Douglas, S. Das, A. Wunderlich and J.F. Sauch. 1987. Encystation and expression of cyst antigens by Giardia lamblia in vitro. Science 235:1040-3.

Gillin, F.D., D.S. Reiner and S.E. Boucher. 1988. Small-intestinal factors promote encystation of Giardia lamblia in vitro. Infect. Immun. 56:705-7.

Gillon, J.D., D. Al Thamery and A. Ferguson. 1982. Features of small intestinal pathology (epithelial cell kinetics, intraepithelial lymphoc tes, disaccharidases) in a primary Giardia muris infection. Gut 23:498-506.

Goding, J.W. 1983. Monoclonal Antibodies: Principles and Practice, 64. Orlando: Academic Press.

Goodman, M.G., and W.O. Weigle. 1979. T cell regulation of polyclonal B cell responsiveness. II. Evidence for a deficit in T cell function in mice with an X-linked B lymphocyte defect. J. Immunol. 123:2484-7.

Hardy, R.R., K. Hayakawa, J. Haaijman and L.A. Herzenberg. 1982. B-cell subpopulations identified by two-color fluorescence analysis. Nature 297:589-91.

Hayakawa, K., R.R. Hardy, L.A. Herzenberg and L.A. Herzenberg. 1985. Progenitors for Ly-1 B cells are distinct from progenitors for other B cells. J. Exp. Med. 161:1554-68.

Hayakawa, K., R.R. Hardy and L.A. Herzenberg. 1986. Peritoneal Ly-1 B cells: Genetic control, autoantibody production, increased lambda light chain expression. Eur. J. Immunol. 16:450-6.

Hayakawa, K., R.R. Hardy, A.M. Stall, L.A. Herzenberg and L.A. Herzenberg. 1986. Immunoglobulin-bearing B cells reconstitute and maintain the murine Ly-1 B cell lineage. Eur. J. Immunol. 16:1313-6.

Herzenberg, L.A., A.M. Stall, P.A. Lafor, C. Sidman, W.A. Moore, D.R. Parks and L.A. Herzenberg. 1986. The Ly-1 B cell lineage. Immunol. Rev. 93:81-102.

Heyworth, M.F., R.L. Owen, W.E. Seaman, F.W. Schaeffer and A.L. Jones. 1985a. Harvesting of leukocytes from intestinal lumen in murine giardiasis and preliminary characterization of these cells. Dig. Dis. Sci. 30:149-53.

Heyworth, M.F., R.L. Owen and A.L. Jones. 1985b. Comparison of leukocytes obtained from the intestinal lumen of Giardia-infected immunocompetant mice and nude mice. Gastroenterol. 89:1360-5.

Heyworth, M.F. 1986. Antibody response to Giardia muris trophozoites in mouse intestine. Infect. Immun. 52:568-71.

Heyworth, M.F., J.R. Carlson and T.H. Ermak. 1987. Clearance of Giardia muris infection requires helper/inducer T lymphocytes. J. Exp. Med. 165:1743-8.

Heyworth, M.F., J.E. Kung and E.C. Eriksson. 1986. Clearance of Giardia muris infection in mice deficient in natural killer cells. Infect. Immun. 54:903-4.

Hill, D.R., E.L. Hewlett and R.D. Pearson. 1981. Lectin binding by Giardia lamblia. Infect. Immun. 34:733-8.

Hill, D.R., J.J. Burge and R.D. Pearson. 1984. Susceptibility of Giardia lamblia trophozoites to the lethal effect of human serum. J. Immunol. 132:2046-52.

Hill, D.R., and R.D. Pearson. 1987. Ingestion of Giardia lamblia trophozoites by human mononuclear phagocytes. Infect. Immun. 55:3155-61.

Hodes, R.J., and A. Singer. 1977. Cellular and genetic control of antibody responses in vitro. I. Cellular requirements for the generation of genetically controlled primary IgM responses to soluble antigens. Eur. J. Immunol. 1:892-6.

Huber, B., R.K. Gershon and H. Cantor. 1977. Identification of a B cell surface structure involved in antigen-dependent triggering: Absence of this structure on B cells from CBA/N mutant mice. J. Exp. Med. 145:10-20.

Huber, B.T. 1982. B cell differentiation antigens as probes for functional B cell subsets. Immunol. Rev. 64:57-79.

Hudson, L., and F.C. Hay. 1980. Practical Immunology, 239. Oxford: Blackwell Scientific Publications (In Canada: Blackwell Mosby Book Distributors, Toronto).

Hunter, K.W., F.D. Finkelman, G.T. Strickland, P.C. Sayles and I. Scher. 1979. Defective resistance to Plasmodium yoelii in CBA/N mice. J. Immunol. 123:133-7.

Inge, P.M.G., G.M.Edson and M.J.G. Farthing. 1988. Attachment of Giardia lamblia to rat intestinal epithelial cells. Gut 29:795-801.

Janeway, C.A., and D.R. Barthold. 1975. An analysis of the defective response of CBA/N mice to T-dependent antigens. J. Immunol. 115:898-900.

Jarroll, E.L., P.J. Muller, E.A. Meyer and S.A. Morse. 1981. Lipid and carbohydrate metabolism of Giardia lamblia. Mol. Biochem. Parasitol. 2:187-96.

Jayawardena, A.N., C.A. Janeway and J.D. Kemp. 1979. Experimental malaria in the CBA/N mouse. J. Immunol. 123:2532-9.

Kanwar, S.S., N.K. Ganguly, B.N.S. Walia and R.C. Mahajan. 1986. Direct and antibody dependent cell mediated cytotoxicity against Giardia lamblia by splenic and intestinal lymphoid cells in mice. Gut 27:73-7.

Kaplan, B.S., S. Uni, M. Aikawa and A.A.F. Mahmoud. 1985. Effector mechanism of host resistance in murine giardiasis: Specific IgG and IgA cell-mediated toxicity. J. Immunol. 134:1975-81.

Kaye, H.S., W.R. Dowdle and J.L. McQueen. 1969. Studies on inactivated influenza vaccines. I. The effect of dosage on antibody response and protection against homotropic and heterotropic influenza virus challenge in mice. Am. J. Epidemiol. 90:162-9.

Kenny, J.J., G. Guelde, J.L. Claflin and I Scher. 1981. Altered idiotype response to phosphocholine in mice bearing an X-linked immune defect. J. Immunol. 127:1629-33.

Kincaide, P.W., H. Jyonouchi, K.S. Landreth and G. Lee. 1982. B lymphocyte precursors in immunodeficient, autoimmune and anemic mice. Immunol. Rev. 64:81-98.

Kiyono, H., L.M. Mosteller, J.H. Eldridge, S.M. Michalek and J.R. McGhee. 1983. IgA responses in Xid mice: Oral antigen primes Peyer's patch cells for in vitro immune responses and secretory antibody production. J. Immunol. 131:2616-22.

Kroese, F.G.M., A.M. Stall, E.C. Butcher and L.A. Herzenberg. 1989. A major peritoneal reservoir of precursors for intestinal IgA-plasma cells. Immunol. Invest. 18:47-58.

Kumkum, R,K., M. Khuller, S. Mehta and V.K. Vinayak. 1988. Plasma membrane associated antigens of trophozoites of axenic Giardia lamblia. Trans. Roy. Soc. Trop. Med. Hyg. 82:439-44.

Laemmli, U.K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227:680-5.

LeFevre, M.E., R. Hammer and D.D. Joel. 1982. Macrophages of the mammalian small intestine: A review. J. Reticuloendoth. Soc. 26:553-73.

Letvin, N.L., B.T. Huber and B. Benacerraf. 1982. B lymphocyte responses to trinitrophenyl-Ficoll. II. Decreased splenic adherent cell function in mice expressing the Xid gene. Cell. Immunol. 70:241-7.

Lev, B., H. Ward, G.T. Keusch and M.E.A. Pereira. 1986. Lectin activation in Giardia lamblia by host protease: A novel host-parasite interaction. Science 232:71-3.

Loftness, T.J., S.L. Erlandsen, I.D. Wilson and E.A. Meyer. 1984. Occurrence of specific secretory immunoglobulin A in bile after inoculation of Giardia lamblia trophozoites into rat duodenum. Gastroenterol. 87:1022-9.

Lucas, S.J., D.W. Barry and P. Kind. 1978. Antibody production and protection against influenza virus in immunodeficient mice. Infect. Immun. 20:115-9.

MacDonald, H.R. 1987. Separation and characterization of subpopulations of T cells. In Cell Separation: Methods and Selected Applications, ed. T.G. Pretlow and T.P. Pretlow, 103-18. Orlando: Academic Press.

MacDonald, T.T. and A. Ferguson. 1978. Small intestinal epithelial cell kinetics and protozoal infection in mice. Gastroenterol. 74:496-500.

Magnani, J.L., M. Brockhaus, D.F. Smith, V. Ginsburg, M. Balszczyk, K.F. Mitchell, Z. Steplewski and H. Koprowski. 1981. A monosialoganglioside is a monclonal antibody-defined antigen of colon carcinoma. Science 212:55-6.

Marshak-Rothstein, A., P. Fink, T. Gridley, D.H. Raulet, M.J. Bevan and M.L. Gefter. 1979. Properties and applications of monoclonal antibodies directed against determinants of the Thy-1 locus. J. Immunol. 122:2491-7.

McDermott, M.R., and J. Bienenstock. 1979. Evidence for a common mucosal immunologic system. I. Migration of B immunoblasts into intestinal, respiratory and genital tissues. J. Immunol. 122:1892-8.

Mendenhall, W. 1979. Introduction to Probability and Statistics, 5th ed., North Scituate MA: Duxbury Press.

Meyer, E.A. 1985. The epidemiology of giardiasis. Parasitol. Today 1:101-5.

Mishell, B.B., M.M. Shiigi, C. Henry, E.L. Chan, J. North, R. Gallily, M. Slomich, K. Miller, J. Marbrook, D. Parks and A.H. Good. 1980. Preparation of mouse cell suspensions. In Selected Methods in Cellular Immunology, ed. B.B. Mishell and M.M. Shiigi, 3-27. San Francisco: W.H. Freeman and Co.

Mitchell, D.M., A.J. McMichael and J.R. Lamb. 1985. The immunology of influenza. Br. Med. Bull. 41:80-5.

Moore, G.T., W.M. Cross, D. McGuire, C.S. Mollohan, N.N. Gleason, G.R. Healy and L.H. Newton. 1969. Epidemic giardiasis at a ski resort. New Engl. J. Med. 281:402-7.

Nair, K.V., J. Gillon and A. Ferguson. 1981. Corticosteroid treatment increases parasite numbers in murine giardiasis. Gut 22:475-80.

Nash, T.E., and A. Aggarwal. 1986. Cytotoxicity of monoclonal antibodies to a subset of Giardia isolates. J. Immunol. 136:2628-32.

Nash, T.E., D.A. Herrington, G.A. Losonsky and M.M. Levine. 1987. Experimental human infections with Giardia lamblia. J. Inf. Dis. 156:974-84.

O'Brien, A.D., I. Scher, G.H. Campbell, R.P. McDermott and S.B. Formal. 1979. Susceptibility of CBA/N mice to infection with Salmonella typhimurium: Influence of the X-linked gene controlling B lymphocyte function. J. Immunol. 123:720-4.

O'Brien, A.D., I. Scher and E.S. Metcalf. 1981. Genetically conferred defect in anti-salmonella antibody formation renders CBA/N mice innately susceptible to Salmonella typhimurium infection. J. Immunol. 126:1368-72.

Olveda, R.K., J.S. Andrews and E.L. Hewlett. 1982. Murine giardiasis: Localization of trophozoites and small bowel histopathology during the course of infection. Am. J. Trop. Med. Hyg. 31:60-6.

Ono, S., L.J. Yaffe, J.L. Ryan and A. Singer. 1983. Functional heterogeneity of the Lyb 5 B cell subpopulation: Mutant Xid B cells and normal Lyb 5 B cells differ in their responsiveness to phenol-extracted lipopolysaccharide. J. Immunol. 130:2014-21.

Osterholm, M.T., J.C. Forfang, T.L. Ristinen, A.G. Dean, J.W. Washburn, J.R. Godes, R.A. Rude and J.G. McCullough. 1981. An outbreak of foodborne giardiasis. New Engl. J. Med. 304:24-8.

Owen, R.L., P.C. Nemanic and D.P. Stevens. 1979. Ultrastructural observations on giardiasis in a murine model. I. Intestinal distribution, attachment and relationship to the immune system of Giardia muris. Gastroenterol. 76:757-68.

Owen, R.L., C.L. Allen and D.P. Stevens. 1981. Phagocytosis of Giardia muris by macrophages in Peyer's patch epithelium in mice. Infect. Immun. 33:591-601.

Paige, C.J., P.W. Kincaide, M.A.S. Moore and G. Lee. 1979. The fate of fetal and adult B cell progenitors grafted into immunodeficient CBA/N mice. J. Exp. Med. 150:548-63.

Perlmutter, R.M., M. Naum, K.E. Stein, J. Slack, I. Zitron, W.E. Paul and J.M. Davie. 1979. Immunoglobulin subclass-specific immunodeficiency in mice with an X-linked B lymphocyte defect. J. Exp. Med. 149:993-8.

Pickering, L.K. 1985. Problems in diagnosing and managing giardiasis. Ped. Inf. Dis. 4:S6-10.

Pilarski, L.M., and A.J. Cunningham. 1975. Host-derived, antibody-forming cells in lethally-irradiated mice. J. Immunol. 114:138-40.

Quan, Z.S., F.F. Dick, B. Regueiro and J. Quintans. 1981. B cell heterogeneity. II. Transplantation resistance in Xid mice which affects the ontogeny of B cell subpopulations. Eur. J. Immunol. 11:643-9.

Quintans, J., and R.B. Kaplan. 1978. Failure of CBA/N mice to respond to thymus-dependent and thymus-independent phosphorylcholine antigens. Cell. Immunol. 38:294-301.

Quintans, J., J.P. McKearn and D. Kaplan. 1979. B cell heterogeneity. I. A study of B cell subpopulations involved in the reconstitution of an X-linked immune defect of B cell differentiation. J. Immunol. 122:1750-6.

Radulescu, S., and E.A. Meyer. 1981. Opsonization in vitro of Giardia lamblia trophozoites. Infect. Immun. 32:852-6.

Reale, M.A., C.A. Bona and J.L. Schulman. 1985. Isotype profiles of anti-influenza antibodies in mice bearing the Xid defect. J. Virol. 53:425-9.

Reiner, D.S., H. Douglas and F.D. Gillin. 1989. Identification and localization of cyst-specific antigens of Giardia lamblia. Infect. Immun. 57:963-8.

Roberts-Thomson, I.C., D.P. Stevens, A.A.F, Mahmoud and K.S. Warren. 1976a. Giardiasis in the mouse: An animal model. Gastroenterol. 71:57-61.

Roberts-Thomson. I.C., D.P. Stevens, A.A.F. Mahmoud and K.S. Warren. 1976b. Acquired resistance to infection in an animal model of giardiasis. J. Immunol. 117:2036-7.

Roberts-Thomson, I.C., and G.F. Mitchell. 1978. Giardiasis in mice. I. Prolonged infections in certain mouse strains and hypothymic (nude) mice. Gastroenterol. 75:42-6.

Roberts-Thomson, I.C., and G.F. Mitchell. 1979. Protection of mice against G. muris infection. Infect. Immun. 24:971-3.

Rosenstreich, D.L., S.N. Vogel, A. Jacques, L.M. Wahl, I. Scher and S.E. Mergenhagen. 1978. Differential endotoxin sensitivity of lymphocytes and macrophages from mice with an X-linked defect in B cell maturation. J. Immunol. 121:685-90.

Rosenwasser, L.J., and B.T. Huber. 1981. The Xid gene controls Ia-W39-associated immune response gene function. J. Exp. Med. 153:1113-23.

Scher, I., M.M. Frantz and A.D. Steinberg. 1973. The genetics of the immune response to a synthetic double-stranded RNA in a mutant CBA mouse strain. J. Immunol. 110:1396-401.

Scher, I., A. Ahmed, D.M. Strong, A.D. Steinberg and W.E. Paul. 1975a. X-linked B lymphocyte immune defect in CBA/HN mice. I. Studies of the function and composition of spleen cells. J. Exp. Med. 141:788-803.

Scher, I., A.D. Steinberg, A.K. Berning and W.E. Paul. 1975b. X-linked B lymphocyte defect in CBA/N mice. II. Studies of the mechanisms underlying the immune defect. J. Exp. Med. 142:637-50.

Scher, I., A.K. Berning and R. Asosfsky. 1979. X-linked B lymphocyte defect in CBA/N mice. IV. Cellular and environmental influences on the thymus-dependent IgG anti-sheep red blood cell response. J. Immunol. 123:477-86.

Scher, I. 1982a. The CBA/N mouse strain: An experimental model illustrating the influence of the X chromosome on immunity. Adv. Immunol. 31:1-71.

Scher, I. 1982b. CBA/N immune defective mice: Evidence for the failure of a B cell subpopulation to be expressed. Immunol. Rev. 64:117-36.

Sieckmann, D.G., R. Asofsky, D.E. Mosier, I.M. Zitron and W.E. Paul. 1978a. Activiation of mouse lymphocytes by anti-immunoglobulin. I. Parameters of the proliferative response. J. Exp. Med. 147:814-29.

Sieckmann, D.G., I. Scher, R. Asofsky, D.E. Mosier and W.E. Paul. 1978b. Activation of mouse lymphocytes by anti-immunoglobulin. II. A thymus-independent response by a mature subset of B lymphocytes. J. Exp. Med. 148:1628-43.

Slack, J., G.P. Der-Balian, M. Nahm and J.M. Davie. 1980. Subclass restriction of murine antibodies. II. The IgG placque-forming cell response to thymus-independent type 1 and type 2 antigens in normal mice and mice expressing an X-linked immunodeficiency. J. Exp. Med. 151:853-62.

Smith, P.D., D.B. Keister, C.O. Elson. 1983. Human host response to Giardia lamblia. II. Antibody-dependent killing in vitro. Cell. Immunol. 82:308-15.

Smith, P.D., D.B. Keister, S.M. Wahl and M.S. Meltzer. 1984. Defective spontaneous but normal antibody-dependent cytotoxicity for an extracellular protozoan parasite, Giardia lamblia, by C3H/HeJ mouse macrophages. Cell. Immunol. 85:244-51.

Smith, H.R., L.J. Yaffe, T.M. Chused, E.S. Raveche, D.M. Klinman and A.D. Steinberg. 1985. Analysis of B cell subpopulations. I. Relationships among splenic Xid, Ly 1+, and Lyb 5+ B cells. Cell. Immunol. 92:190-6.

Snider, D.P. 1985. Evidence for the requirement of B cells and antibody in the clearance of Giardia muris infection in mice. Ph.D. diss., University of Toronto.

Snider, D.P., J. Gordon, M.R. McDermott and B.J. Underdown. 1985. Chronic Giardia muris infection in anti-IgM-treated mice. I. Analysis of immunoglobulin and parasite-specific antibody in normal and immunoglobulin-deficient animals. J. Immunol. 134:4153-62.

Snider, D.P., and B.J. Underdown. 1986. Quantitative and temporal analyses of murine antibody response in serum and gut secretions to infection with Giardia muris. Infect. Immun. 52:271-8.

Snider, D.P., D. Skea and B.J. Underdown. 1988. Chronic giardiasis in B cell deficient mice expressing the Xid gene. Infect. Immun. 56:2838-42.

Sprent, J., and J. Bruce. 1984. Physiology of B cells in mice with X-linked immunodeficiency (Xid). III. Disappearance of Xid B cells in double bone marrow chimeras. J. Exp. Med. 160:711-23.

Sprent, J., J. Bruce, Y. Ron and S.R. Webb. 1985. Physiology of B cells in mice with X-linked immunodeficiency. I. Size, migratory properties and turnover of the B cell pool. J. Immunol. 134:1442-8.

Stein, K.E., C.A. Brennan, J.J. Mond, O. Makela and W.E. Paul. 1980. Antibody affinity in mice with the CBA/N defect. J.Immunol. 124:1798-802.

Stevens, D.P., and D.M. Frank. 1978. Local immunity in murine giardiasis: Is milk protective at the expense of maternal gut? Trans. Assoc. Am. Physicians 91:268-72.

Stevens, D.P., D.M. Frank and A.A.F. Mahmoud. 1978. Thymus dependency of host resistance to Giardia muris infection: Studies in nude mice. J. Immunol. 120:680-2.

Subbarao, B., A. Ahmed, W.E. Paul, I. Scher, R. Lieverman and D.E. Mosier. 1979. Lyb 7, a new B cell alloantigen controlled by genes linked to the IgC_H locus. J. Immunol. 122:2279-85.

Towbin, H., T. Staehlin and J. Gordon. 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: Procedure and some applications. Proc. Natl. Acad. Sci. (USA) 76:350-6.

Udhayakumar, V., S.N. Goud and B. Subbarao. 1988. Physiology of murine B lymphocytes. I. Life spans of anti-mu and haptenated Ficoll (thymus-independent antigen)-reactive B cells. Eur. J. Immunol. 18:1593 9.

Underdown, B.J., I.C. Roberts-Thomson, R.F. Anders and G.F. Mitchell. 1981. Giardiasis in mice: Studies on the characteristics of chronic infection in C3H/He mice. J. Immunol. 126:669-72.

Underdown, B.J., D.L. Skea, G.M. Loney and D.P. Snider. 1988. Murine giardiasis and mucosal immunity: A model for the study of immunity to intestinal protozoan parasites. Monogr. Allergy 24:287-96.

Vioque, E. 1984. Spray reagents for thin layer chromatography (TLC) and paper chromatrography (PC). In CRC Handbook of Chromatography, Lipids, Volume II, ed. H.K. Mangold, 314. Cleveland: CRC Press.

Volf, D., L.L. Sensenbrenner, S.J. Sharkis, G.J. Elfenbein and I. Scher. 1978. Induction of partial chimerism in non-irradiated B lymphocyte deficient CBA/N mice. J. Exp. Med. 147:940-5.

Waldmann, J.A., and W. Strober. 1969. Metabolism of immunoglobulins. Prog. Allergy 13:1-110.

Ward, H.D., B.I. Lev, A.V. Kane, G.T. Keusch and M.E.A. Pereira. 1987. Identification and characterization of taglin, a mannose-6-phosphate binding, trypsin-activated lectin from Giardia lamblia. Biochem. 26:8669-75.

Ward, H.D., J. Alroy, B.I. Lev, G.T. Keusch and M.E.A. Pereira. 1988. Biology of Giardia lamblia. Detection of N-acetyl-D-glucosamine as the only surface saccharide moiety and identification of two distinct subsets of trophozoites by lectin binding. J. Exp. Med. 167:73-88.

Webb, S.R., D.E. Mosier, D.B. Wilson and J. Sprent. 1984. Negative selection in vivo reveals expression of strong Mls determinants in mice with X-linked immunodeficiency. J. Exp. Med. 160:108-15.

Wysocki, L.J., and V.L. Sato. 1978. Panning for lymphocytes: A method for cell selection. Proc. Natl. Acad. Sci. (USA) 75:2844-8.

Zweerink, H.J., M.C. Gammon, C.F. Hutchinson, J.J. Jackson, G.B. Pier, J.M. Puckett, T.J. Sewell and N.H. Sigal. 1988. X-linked immunodeficient mice as a model for testing the protective efficacy of monoclonal antibodies against Pseudomonas aeruginosa. Infect. Immun. 56:1209-14.