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THE EFFECTS OF ERYTHROCYTE ALLOANTIGEN L ON THE AVIAN IMMUNE RESPONSE

BY

ZDRAVKA OGNYANOVA MEDAROVA

B.A., UNIVERSITY OF SOUTHERN MAINE, 1998

DISSERTATION

Submitted to the University of New Hampshire in Partial Fulfillment of the Requirements for the Degree of

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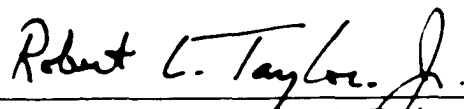
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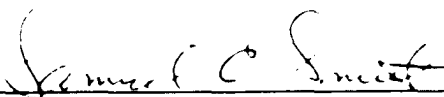
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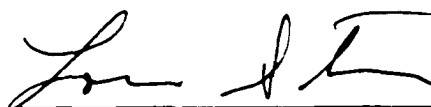
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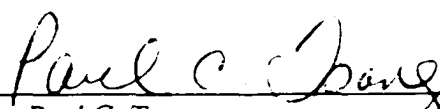
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DEDICATION

To my family

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I would like to thank my advisor, Dr. Robert L. Taylor, Jr., for his guidance and patience without which this work would not have been possible. I would also like to thank Dr. John J. Collins for his excellent instruction, to which I was exposed in the classes I took with him. I would like to thank Dr. Samuel C. Smith, Dr. Louis S. Tisa, and Dr. Paul C. Tsang for their invaluable advice. I would like to express my gratitude to Dr. W.E. Briles and Dr. Keith T. LePage, whose work represents the foundation for this study. I am deeply indebted to my friends, family, and co-workers from the Taylor Lab for their moral and physical support and for their sense of humor, which is crucial for the completion of any long-term effort.

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ABSTRACT

THE EFFECTS OF ERYTHROCYTE ALLOANTIGEN L ON THE AVIAN IMMUNE RESPONSE

BY

Zdravka O. Medarova

University of New Hampshire, December 2002

Experiment 1 examined the alloantigen system L effects on Rous sarcomas in three B complex genotypes. The parental stock were 50% Modified Wisconsin Line 3 and 50% inbred Line 6.15-5. B^2B^5 L^1L^2 x B^2B^5 L^1L^2 matings produced experimental chicks. Chicks were inoculated with 20 pock-forming units (pfu) Rous sarcoma virus (RSV) at 6 weeks-of-age. Tumors were scored six times over 10 weeks postinoculation. Tumor scores were used to assign a tumor profile index (TPI) to each chicken. Results were evaluated by ANOVA. The B complex affected the responses. Separate analyses revealed L system effects ($P < 0.05$) only in B^5B^5 chickens.

Experiment 2 examined the influence of $Ea-L$ on antibody response to SRBC and *Brucella abortus* (BA). The mating protocol was the same as in Experiment 1. At 4 and

11 weeks of age the experimental birds were injected intravenously with standard concentrations of SRBC and BA. Total and ME-resistant antibody titers were determined as described. Results were analyzed by ANOVA. $Ea-L$ had an effect on total primary antibody titer to SRBC in a B^sB^s background (p < 0.004) and on total (p < 0.011) and ME-resistant (p < 0.017) secondary titer to SRBC in a B^2B^s genotypic background. $Ea-L$ also affected total (p < 0.004) and ME-resistant (p < 0.005) secondary titer to $Brucella$ $abortus$ in a B^2B^s background.

Experiment 3 examined the effect of $Ea-L$ on resistance and acquired immunity to $E. tenella$ infection. The mating protocol was the same as in Experiments 1 and 2. In the resistance and susceptibility study, chicks were weighed and inoculated with 30,000 $E.$ $tenella$ oocysts at 6 weeks of age. 6 days post-inoculation, the birds were weighed again and assigned a cecal lesion score. In the immunity study, the challenge procedure was preceded by inoculations of 500 oocysts per day beginning at 5 weeks. Weight gain and cecal lesion scores were evaluated by ANOVA. The B complex affected lesion score in the immunity but not the resistance and susceptibility study and did not affect weight gain in either study. The L system had no effect in either study.

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CHAPTER I

LITERATURE REVIEW

Antigen Presentation

The complexity of the immune response is very narrowly defined by the need to satisfy two seemingly contradictory requirements. On the one hand, the immune system has to be able to recognize and annihilate the immense variety of foreign antigens that its host organism encounters in its lifetime. On the other hand, the immune system has to identify all of the antigens expressed on the surface of host cells as "self" and develop tolerance to them. Failure to satisfy these requirements would lead to either unresponsiveness or autoimmunity, and consequently harm the host organism.

The high level of conservation of molecular structures between organisms, extending both from functional constraints and common ancestry, makes the task of achieving immune responsiveness to a great variety of foreign antigens without concomitant autoimmunity appear impossible. The key element of the solution to this problem lies in the process of antigen presentation. The different levels of the antigen presentation machinery are very carefully coordinated through a series of checkpoints and signaling thresholds, and through the need for co-stimulation, and division of labor among different cell types. The final result is a fine-tuned network of continuously modulated responses which alerts the immune system to the presence of foreign or altered "self" antigens as well as to the nature and quantity of these

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antigens, in order to trigger the optimal series of events leading to the elimination of these antigens without damage to "self".

Basic Overview of Antigen Presentation

Two types of cells define antigen presentation: antigen presenting cells (APCs), i.e., macrophages and dendritic cells (DCs), and lymphocytes, such as B cells and T cells. Antigen presenting cells (APCs) trap, concentrate, and present exogenous antigen on their surface in the context of MHC molecules. If presented in the context of MHC II, antigen is recognized by CD4$^+$ T-helper (T_h) cells. That recognition activates the T cell to proliferate and release cytokines necessary for the activation of antigen-specific effector cells. These effector cells include B and T cells that generate a humoral or cell-mediated immune response, respectively, as well as nonspecific effector cells such as natural killer cells (NK) and macrophages.

Endogenous antigens can be presented in the context of MHC I molecules which are expressed on all nucleated cells of the body. In that context, these antigens can be recognized by CD8$^+$ T-cytotoxic (T_c) cells. This cytotoxic action mediates membrane damage to altered self cells and causes them to lyse.

Concomitant cross-linking of the B cell receptor (BCR) by antigen is necessary for the B cell to respond to the activation signals released by T_h cells in response to antigen recognition. Successful activation of T_c cells by MHC-I-associated antigen requires the presence of growth factor signals released by activated T_h cells. The necessity of at least two signals, one through antigen binding to the effector cell and one through cytokines released by T_h cells, is an example of the complex immune interactions, evolved to safeguard against immune dysregulation (Kuby, 1992).

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Role of Inflammation in Antigen Presentation

Inflammation not only provides the first line of defense against infection but also supplies the first signals necessary to stimulate antigen presentation. Macrophages and dendritic cells (DCs) respond to the recognition of "foreign-ness" by increased proliferation and differentiation. Examples of entities to which macrophages and DCs respond are: elements of bacterial cell walls, such as lipopolysaccharide (LPS) and peptidoglycan, as well as bacterial DNA and double-stranded viral RNA. Furthermore, complement, which is an element of the innate immune system, as well as opsonizing antibody, tag a particle as foreign and thus provide further signals to professional APCs. In addition, inflammatory cytokines, such as TNF-α and IL-1, released by macrophages and DCs in response to foreign entity recognition, identify an environment as an immune response target, and initiate a cascade of events leading to immune cell influx and activation (Siemasko and Clark, 2001).

Role of Macrophages and Dendritic Cells in Antigen Presentation

Both macrophages and dendritic cells are believed to be phagocytic cells (Watts and Amigorena, 2001). The processes involved in antigen presentation by macrophages and dendritic cells are very similar with few exceptions. Dendritic cells are believed to be the initiators of all T cell responses (Banchereau et al., 2000). They are located at body surfaces (e.g., the skin) where they encounter antigens at their point of entry into the host organism. Furthermore, DCs appear superior to macrophages and B cells in the co-stimulation of naïve T cells. This property is a function of the capacity of DCs to undergo "maturation" as a result of antigen capture and up-regulate accessory molecule expression (Nussenzweig et al., 1980). In this sense, dendritic cells are "sentinels"

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priming the immune system (Mellman et al., 1998). Activation of DCs occurs in response to direct recognition of microbial products, such as LPS, inflammatory cytokines, chemokines, or tissue damage (Reis e Sousa, 1999).

Inflammation signals local tissue dendritic cells to migrate to lymph nodes following antigen capture. During migration, DCs go through a process of maturation, which is associated with increased MHC-antigen display on the cell surface and synthesis of co-stimulatory and adhesion molecules, such as B7, necessary for efficient T cell activation. Remarkably, the process of maturation also involves the discontinuation of further phagocytosis to prevent the presentation of irrelevant antigens encountered during the process of migration (Watts and Amigorena, 2001). After arriving in the T cell area of a lymph node, DCs present MHC-antigen complexes to naïve T cells (Jenkins et al., 2001). In addition to presenting antigen, DCs also release cytokines which stimulate T cell proliferation, e.g., IL-12, and provide co-stimulation, e.g., through an interaction between B7 on the APC and CD28 on the T cell. This provides a second activation signal and also allows inflammatory cytokines to mediate expansion of antigen-stimulated T cells (Jenkins et al., 2001). Once activated, T cells can then re-circulate through the body and signal effector cells, such as B cells, macrophages, and cytotoxic T cells to become activated (Mellman et al., 1998).

Traditionally, presentation in the context of MHC I to CD8⁺ T cells has only been considered relevant to endogenous antigens. These are altered-self antigens or viral antigens which gain access to the cytoplasmic compartment of the cell. Recent evidence has emerged that exogenous antigens, e.g., peptides released from apoptotic cells, also prime MHC-I-restricted cytotoxic T cell responses (Peppelenboch et al., 2000). This

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phenomenon is known as "cross-presentation". Cross-presentation of exogenous antigens to CD8$^+$ T cells allows professional APCs, such as macrophages and DCs, which acquire antigen in nonlymphoid tissues, to present these antigens to naïve T cells in lymphoid tissues. This process permits T cells to scan the host organism for pathogens without having to migrate outside of the lymph nodes (Kurts C., 2000).

Macrophages have inferior antigen presenting capacity to that of DCs. Macrophages are also less efficient at activating naïve T cells (Pieters J., 2000) because they are located outside of the T cell areas of lymph nodes. DCs are located within the T cell areas of lymph nodes where naïve T cells are physically restricted (Jenkins et al., 2001). Even though macrophages appear to be capable of handling a broader range of phagocytic substrates than DCs (Austyn, 1996), phagocytosis by DCs triggers a sequence of maturation events in DCs, such as up-regulated MHC II expression and active migration into T cell areas of secondary lymphoid organs, which enhance the antigen presenting efficiency of DCs (Watts and Amigorena, 2001). In fact, it has been shown that monocytes can differentiate into DCs as a result of phagocytosis (Randolph et al., 1998).

Still the processes involved in macrophage activation and antigen presentation are similar to the ones involved in presentation of antigens by DCs. Macrophages are activated and induced to migrate to lymph nodes by inflammatory cytokines, particularly IFN-γ. Activated macrophages, which have phagocytosed antigen, present that antigen in the context of MHC to T cells and produce T cell activating cytokines, such as IL-12 (Martin-Orozko et al., 2001), leading to T cell activation and proliferation.

Role of B cells in Antigen Presentation

While resting B cells have been considered poor antigen presenters due to their low expression of co-stimulatory molecules, activated B cells are capable of presenting antigen to naive T cells. The distinguishing feature of B cells as APCs is their capacity to stimulate T cells in an antigen-specific manner through antigen capture by the BCR (Bar-Or et al., 2001). It is believed that B cells form a second line of antigen presentation after DCs. Following antigen capture and concentration on the surface of DCs, B cells are believed to gain access to that antigen for antigen specific presentation to T cells (Batista et al., 2000). In this sense, B cells are considered antigenic reservoirs which have the potential to amplify and fine-tune T cell responses by "selecting" antigenic epitopes for presentation to T cells based on their immunogenicity and abundance. This mechanism of antigen capture by B cells also allows them to recognize and acquire rare low-affinity antigens which drive primary acquired responses (Siemasko and Clark, 2001). In this way, B cells provide a transition between the presentation of random antigenic epitopes associated with the innate immune system and defined by macrophages and DCs, and the presentation of very specific rare epitopes associated with the acquired immune system. By completing this transition, the immune response is channeled towards very selective targets linked to the invading pathogen and away from potential "self" antigens.

B cells are believed to diversify the immune response, in addition to amplifying T cell responses. Presentation of immunodominant epitopes by nonspecific DCs primes a small population of naïve T cells, which then activate antigen-specific B cells. These antigen-specific B cells are capable of internalizing antigen much more efficiently than nonspecific APCs and thus are capable of presenting a greater range of peptides from the

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original protein or proteins associated with it to naïve T cells. Furthermore, sites on an antigen bound by surface immunoglobulin have been shown to be protected from proteolysis. Proteolysis is a prerequisite for presentation of epitopes. Therefore, protection from proteolysis prevents the same epitopes from being presented and mediates the diversification of the peptide repertoire introduced to T cells. By increasing the repertoire of peptides presented to T cells, B cells promote the recruitment of diverse populations of T cells following DC priming of a few T cell clones specific to immunodominant peptides, and in this way diversify the immune response (Mamula and Janeway, 1993).

Following antigen capture, B cells migrate to germinal centers where they proliferate, undergo isotype switching, and develop high affinities for captured antigen. Inside germinal centers B cell receptor (BCR) affinities are checked for optimal access to antigen in competition with other B cells. This initial encounter of antigen through the BCR triggers the first line of signaling events necessary for the B cell to reenter the cell cycle and up-regulate inducible co-stimulatory molecules, such as B7.1 and B7.2. A second line of stimuli is provided following migration of B cells to the T cell areas of the lymph node, where B cells present captured antigen to cognate T cells, i.e. T cells already activated by related antigen epitopes through and interaction with professional APCs (Siemasko and Clark, 2001).

As the immune response proceeds, the influence on antigen presentation begins to shift away from DCs and towards B cells. After antigen stimulation, B cells appear to be present in larger numbers in lymphoid tissues than macrophages and DCs as the numbers

of DCs decrease and their phagocytic abilities are down-regulated (Banchereau and Steinman, 1998).

<u>Two-step, Two-signal Model for the Primary Activation of T_h Cells</u> (Bretscher, 1999)

Contemporary models for the activation of T_h cells describe the process of antigen presentation as an integrated network of interactions between less specific, constitutive co-stimulator APCs, e.g., DCs and macrophages, and antigen-specific inducible co-stimulator APCs, e.g., B cells, and T cells. The first step of the process of precursor T_h cell activation involves the interaction with APCs which present a particular antigen Q and constitutively express co-stimulatory molecules, such as B7. As a result of this interaction, the stimulated T_h cells become primed with antigen and proliferate.

The second step of the process of precursor T_h cell activation involves the interaction of the antigen-primed T_h cell with antigen Q-specific APCs, e.g., B cells which have been activated following binding between the B cell displaying peptides from antigen Q and an effector T_h cell. The interaction between the antigen Q-displaying B cell and the effector T_h cell leads to induction of co-stimulatory molecule expression on the B cell. These co-stimulatory molecules, e.g., B7, are necessary to provide a second signal to the precursor T_h cell along with antigen presentation, in order to efficiently activate the precursor T_h cell. Thus, in the presence of foreign antigen and proper co-stimulation, the activation of precursor T_h cells would expand the spectrum of effector T_h cells.

One problem associated with this model is the need for antigen Q-specific effector T_h cells in order to activate antigen Q-specific B cells. Bretscher (1999) proposes that a small pool of precursor T_h cells endowed with some basal effector abilities exists neonatally and in the presence of foreign antigens would yield a greater variety of

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effector T_h cells specific for that antigen. These effector functions would not impinge on the process of peripheral self-nonself discrimination, since T_h cells specific for self antigens, which are present at birth, would be inactivated by the mechanisms of self tolerance, and only T_h cells not specific for self antigens would survive and form the pool of T_h cells with the capacity to activate B cells.

Major Histocompatibility Complex

The major histocompatibility complex (MHC) is a polymorphic set of genes found on chromosome 6 in humans, chromosome 17 in mice, chromosome 20 in rats, and chromosome 16 in chickens. The MHC was first identified genetically as a restricting element of the immune response to organ transplants between unrelated individuals (Bach and Sachs, 1987). In 1974, Zinkernagel and Doherty established a role for MHC antigens also as restricting elements of immune response to foreign antigens in the context of self. In immunological parlance, MHC antigens are proteins involved in the restriction of immune responses by virtue of the fact that T cells can only recognize peptides presented to them in the context of MHC molecules (Natarajan et al., 1999).

The classical MHC molecules are divided into two classes, class I and class II. This division is based on their structure, the pathways of their assembly, and their immunological functions defined by the types of T cells with which they interact (Natarajan et al., 1999).

The class I genes code for the α chain of the MHC molecule; the β chain, β-2-microglobulin is encoded by a gene on chromosome 15 in humans. The α chain consists of five domains: three α domains, α-1 and α-2 involved in antigen binding, and α-3 which is an immunoglobulin-like domain, one transmembrane domain, and one

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cytoplasmic domain. The class II genes encode two α and two β chains. Each of the class II α and β chains consists of four domains: α-1 or β-1 comprising the peptide-binding domain, α-2 or β-2 which are immunoglobulin-like domains, the transmembrane region, and the cytoplasmic tail (Klein and Sato, 2000).

MHC Antigen Processing and Presentation

MHC antigens of various specificities are expressed on the surfaces of cells in large numbers. Between 100,000 and 300,000 class I or class II products of each of the classical MHC loci are found on the surface of a single cell. Each MHC antigen presents one peptide. As a result, thousands of different peptides are displayed in the context of MHC molecules. Some of these peptides are more abundant than others. However, the average number of copies of a specific peptide found on the cell surface is 100 (Klein and Sato, 2000). Presentation of large numbers of peptides of a defined specificity, as well as the capacity of a single cell to display a variety of peptides, is a function of the mechanism of antigen processing and presentation. MHC I molecules present peptides derived from intracellular proteins by the endogenous route, while MHC II molecules present peptides from extracellular proteins by the exogenous route.

<u>MHC I Antigen Processing and Presentation</u>. The MHC I α chain domains are translated and glycosylated in the ER. Following translation, the α chain domains assemble with β-2-microglobulin and are retained in the ER until bound to antigenic peptide, which stabilizes their conformation. Peptides binding MHC I molecules are derived from endogenous proteins synthesized on ribosomes within the cytosol. Following their synthesis, these proteins are degraded in the cytosol by multicomponent complexes, called proteases and delivered to the ER (Krensky, 1997). Entry of antigens

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generated in the cytoplasm into the ER is mediated by the peptide transporter, TAP (Hudson and Ploegh, 2002). After proper loading of the MHC I molecule with antigen and stabilization of the complex, the peptide-loaded class I molecules depart from the peptide loading compartment and travel to the plasma membrane via the secretory pathway (Hudson and Ploegh, 2002).

MHC II Antigen Processing and Presentation. MHC II molecules are translated and glycosylated in the ER, following which they bind a transmembrane glycoprotein, the invariant chain (Ii), in the peptide-binding groove of the assembled molecule. This complex leaves the ER and travels to the peptide-loading compartment where the invariant chain is proteolyzed to leave a small fragment, class-II associated invariant chain peptide (CLIP), within the peptide-binding groove. A molecule with a structure similar to the MHC II molecule, called HLA-DM, mediates the release of the CLIP peptide from the peptide-binding groove, which is occupied by the antigenic peptide (Hudson and Ploegh, 2002).

MHC II molecules bind exogenous peptides. Exogenous antigens enter the cell via receptor-mediated endocytosis or pinocytosis, pass through an organelle called early endosome, and enter the peptide-loading compartment, which is a specialized endosome characterized by an acidic pH and a high concentration of proteases. Following assembly of the MHC II-antigen complex, it is transported to the plasma membrane by the secretory pathway (Krensky, 1997).

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Mouse MHC (*H-2*)

Historical Overview. The MHC was first described in mice. In 1916, Little and Tyzzer studied the fate of tumor transplants between mice and showed that a few dominantly expressed genes influenced tumor graft outcome. Further evidence for the effect of genetic factors on transplant fate came from a study done by Bover in 1927, who found that skin transplants between identical twins were not rejected.

The first identification of such genetic factors influencing transplant fate came as a result of experiments done by Gorer (1937), who showed that blood type segregates with resistance and susceptibility to transplantable tumors in mice. Gorer tested backcross animals segregating for the *Fu* gene, previously identified by George Snell at the Jackson Laboratory as segregating with tumor fate, and found that the blood group antigen described by him also co-segregates with *Fu* (Gorer et al., 1948). The *Fu* locus identified by Snell and the locus identified by Gorer were one and the same. That locus was called *H-2*.

Soon after the identification of the *H-2* locus as a determinant of transplant fate, the characterization of that locus revealed a high level of complexity. By 1953, seven alleles segregating at the *H-2* locus had been identified. It was determined that the alleles at that locus were expressed co-dominantly and that crossing over can occur within the locus. Ninety recombinants at that locus were identified by 1980. Furthermore, the occurrence of crossing-over suggested that there are at least two loci within the *H-2* region. These loci were called *H-2K* and *H-2D*. (Snell, 1980).

A second method used to identify *H* loci involved the creation of congenic resistant lines. A line was identified which carried a gene, or group of linked genes, that

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made it resist transplants from its partner inbred strain. Through complementation tests and scans for visible markers present in the introduced segment, new *H* loci were identified (Bailey, 1975).

At the time that linkage studies were employed to identify histocompatibility loci, Gorer focused on the serological approach to *H* locus identification. Through red cell agglutination and the cytotoxic action of iso-antibody plus complement on lymphocytes, Gorer produced antisera which, based on the difference between donor and recipient only at *H-2*, contained only anti-*H-2* antibodies. The reaction patterns between these antisera appeared very complex and were defined by characteristic strain-specific distributions. Thus, each reaction pattern was specific and could be assigned a number.

Through the methods described above it has been demonstrated that there are at least 200 alleles at both the *K* and *D* loci (Duncan et al., 1979). Allotyping specificities mapped to two mutually exclusive regions, one mapping within the *K* region and another within the *D* region. Furthermore, a third allotyping specificity region was identified and called *H-2L*. This new locus is closely linked to the *D* locus (Snell et al., 1976).

Since the discovery of the mouse MHC, a series of studies have led to the characterization of the structure of the *H-2* complex. The mouse MHC consists of five regions: *K*, *I*, *S*, *D*, and *Tla*. These regions produce four classes of antigens: MHC I associated with the *K* and *D* regions, MHC II associated with the *I* region, class III associated with the *S* region, and class IV associated with the *Tla* region. The different classes are differentiated based on their chemical properties and tissue distribution. Class I antigens are expressed on all cells except the early embryo. Class II antigens are

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expressed on professional APCs. Class III expression is found in serum, and class IV expression is found on specific lymphocyte sub-populations (Snell, 1980).

One of the first examples of the association between MHC haplotype and disease came in the 1960s, when Tenant and Snell using congenic resistant strains, demonstrated that cell-free extracts from virally-induced leukemias in strains of a certain MHC haplotype preferentially induce leukemias in mice of the same MHC haplotype and that there is a difference in the degree of resistance by other haplotypes (Tennant, 1965).

Later it was determined that immune response genes (*Ir*) map to the *I* region of *H-2* (McDevitt et al., 1972), as well as the *K* and *D* regions (Snell, 1979). Further support for the role of *H-2* antigens in the immune response came from the observation that T cells recognize not only foreign antigens on the surfaces of cells but also MHC antigens and that the two need to be recognized simultaneously in order to produce an immune reaction, a phenomenon known as MHC restriction (Zinkernagel and Doherty, 1974).

Zinkernagel and Doherty studied the basic aspects of immune defense against viruses in various strains of mice in the 1960s and 1970s. They found that T_c cells from one mouse strain are capable of recognizing and killing virally infected cells from another mouse strain only if the two strains share the same *H-2* variants. In this sense, the essence of T cell recognition of foreign antigens may be seen as detection of violated MHC integrity.

The pioneering studies in mice did not only lead to the identification of the genetic restriction of transplant fate and the characterization of the loci which determine histocompatibility. They also established the essence of the immune uniqueness of

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individuals within a species as a function of their possessing a unique set of transplantation antigens.

H-2 Structure (Fig. 1). The mouse MHC spans between 3 and 4 Mb on chromosome 17 and is divided into three regions encoding class I, class II, and class III molecules. The class I region contains the *H-2K*, *-D*, and *-L* loci which together encompass approximately 30 genes. In addition, the class I region includes loci, such as *Qa, Tla*, and *M*, which encode non-classical MHC molecules. The class II MHC molecules are found in the *H-2I* region which contains the *IA* and *IE* loci, as well as a few genes, such as *Pb* and *Eb2*, for which no protein products have been identified. The class I region is split by the class II and class III regions. The class III region encodes about 20 molecules involved in the complement cascade as well as cytokine products such as TNF and molecules involved in antigen processing (Roitt et al., 1998).

Human MHC (*HLA*)

Historical Overview. The human MHC is termed human leukocyte antigen (*HLA*). It contains more than 200 genes, about 40 of which encode antigens expressed on leukocytes. The rest are genes not evolutionarily related to the HLA genes and some are not involved in immunity (Klein and Sato, 2000).

The *HLA* was the third MHC identified after the identification of the mouse *H-2* and the chicken *B* complex. It was discovered independently by several researchers. Dausset described it in 1953 as the MAC antigen based on the observation that sera from transfused patients agglutinated leukocytes (Dausset, 1958). In 1958, Rose Payne identified similar antibodies in sera from multiparous women and named the causative antigen LA2 (Payne et al., 1964). van Rood described the antigen as the 8a antigen and

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Figure 1. MHC gene structure in mouse, human, and chicken

MOUSE H-2 COMPLEX

HUMAN HLA COMPLEX

CHICKEN B COMPLEX

■ CLASS I   ■ CLASS II   ■ CLASS III   CLASS IV

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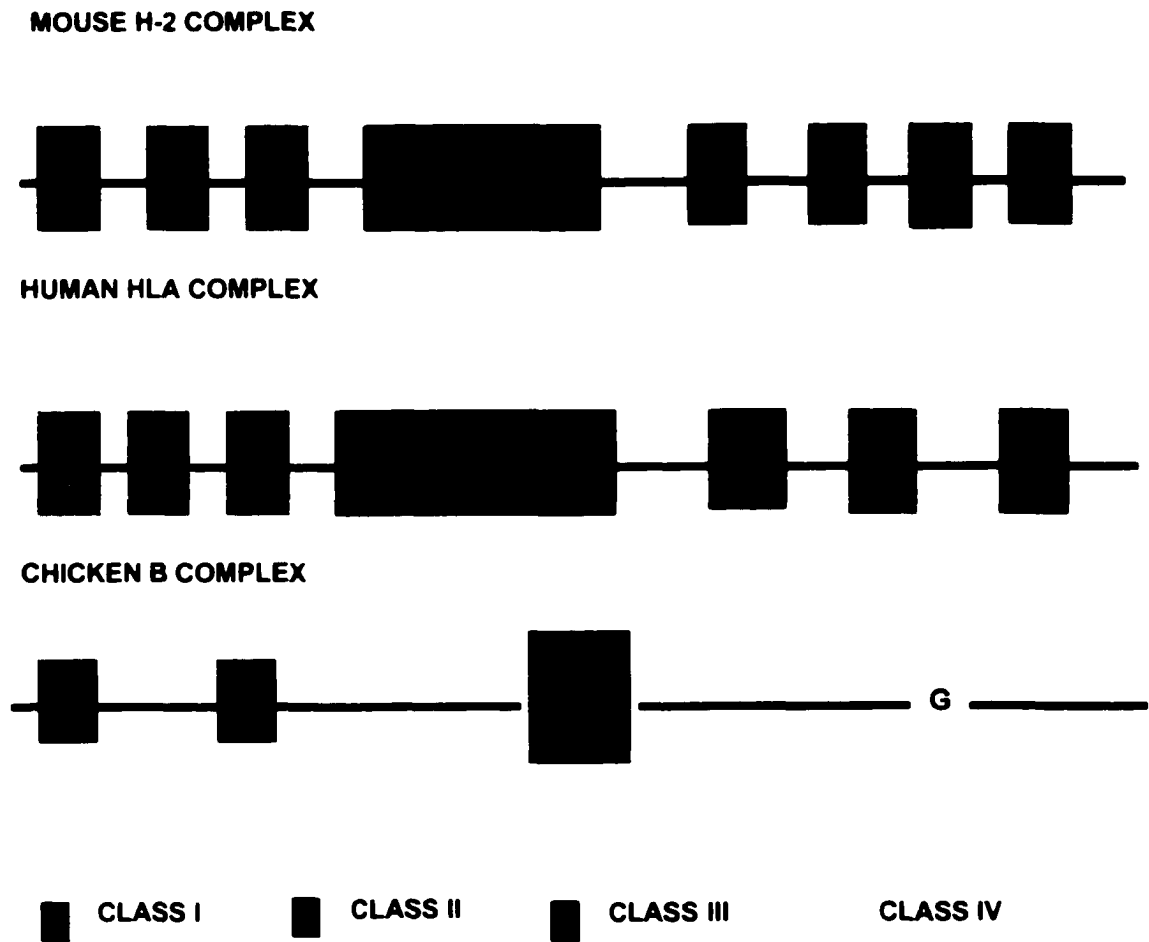
developed computer programs for antigen grouping based on reaction specificity (van Rood, 1965). Since the 1953 report by Bernard Amos that H-2 antigens can be detected using leukoagglutinins, it was suspected that human leukocyte agglutinins played a role in transplantation.

These independent findings were not coordinated until the Second International Histocompatibility Workshop in 1965, when a single panel of 45 cells was tested by different laboratories, using techniques independently developed by these laboratories, to compare agglutinin specificities (Park and Terasaki, 2000). The Third Workshop in 1967, established the presence of two distinct specificities, now known as HLA-A and HLA-B. These specificities were given the common name HLA at the Histocompatibility Organization nomenclature meeting held in 1968.

Following that meeting, the nomenclature of the HLA has evolved in accordance with the improved understanding of the complexity of the system. By 1970, an additional class I locus was identified and named *HLA-C* (Thorsby, 1970). By 1980, the class II loci, *DR*, *DQ*, and *DP*, had been identified (Park, 1978). In 1970, these loci had been described by Amos and Yunis as a genetic system responsible for T-cell activation in mixed lymphocyte culture.

Ever since the discovery in 1967 (Amiel, 1967), that certain MHC polymorphisms are associated with an increased risk of Hodgkin's disease, a series of studies have been designed to find disease associations with specific MHC haplotypes. MHC haplotype has been implicated in the predisposition to a variety of mainly autoimmune or immunopathological conditions, such as viral or bacterial infections (Zinkernagel and Doherty, 1979). So far between 50 and 100 diseases have been linked to *HLA* alleles. For

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some of them, the association with the MHC appears logical, for others it is less clear. Among the diseases listed as linked to particular *HLA* alleles in the Danish HLA and Disease Registry are systemic lupus erythematosus, multiple sclerosis, hemachromatosis, and manic depression.

HLA Structure (Fig. 1). The human MHC spans a region of 6.3 Mb on chromosome 6. It is divided into three regions: class I, class II, and class III. The classical class I loci are *HLA-A*, *HLA-B*, and *HLA-C*. The classical class II loci are *HLA-DP*, *-DQ*, and *-DR*. Other class I genes, *HLA-E*, *-F*, *-G*, *-H*, *-J*, *-K*, and *-L* were recently recognized. Of these, only *HLA-E*, *-F*, and *-G* appear to be expressed. The class III region separates the class I and class II regions on the chromosome. It contains both genes involved in the formation of immune response and genes unrelated to immunity. Some of the genes found in the class III region of the *HLA* are the genes for HSP70, TNF, the complement proteins C4A, C4B, C2, and BF, and *cyp21*, which is the gene for 21-hydroxylase, an enzyme involved in corticosteroid metabolism.

Chicken MHC (*B* complex)

Historical Overview. The chicken *B* complex was the second MHC identified after the mouse MHC and before the human MHC. It was first characterized by serological methods as a polymorphic antigen expressed on chicken erythrocytes (Briles et al., 1950). Antibodies to erythrocyte antigens were generated by injection of whole blood or blood cells into closely related individuals. Test-reagents specific for each antigen were prepared by serum dilution, followed by selective antibody absorption with washed erythrocytes until no agglutination with the cells used for absorption was detected. Selective absorption refers to an initial absorption with cells of birds selected at

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random or because of their erythrocyte antigenic constitution, if it was known, and subsequent testing of the resulting absorbed antisera with the cells of selected birds. The agglutination tests involved mixing of an erythrocyte suspension of unknown antigen genotype with different test-reagents in serial dilutions. This process resulted in the grouping of birds according to the reaction patterns of their erythrocytes with the test-reagents (Briles et al., 1950).

This method led to the identification of thirteen erythrocyte alloantigens, among which was the B system. The same system was also independently identified by Gilmour in 1959 along with another system, named the L system (Gilmour, 1959). The observation of a high level of polymorphism at the B complex even in inbred populations suggested a function for the complex in survival and fitness through a mechanism associated with a selective advantage of heterozygosity at that locus. Schierman and Nordskog elucidated the precise immune role of the B complex in 1961, after they established that the B system controls tolerance to skin homografts (Schierman and Nordskog, 1961). This finding identified the B complex as a determinant of histocompatibility and suggested that it represents the chicken homologue of the mouse MHC.

Soon after the discovery of the chicken MHC, it became clear that at the genetic level, the B system is a complex of at least three loci (Pink et al., 1977). This conclusion was based both on biochemical analysis of the B system antigens and on the first observations of recombination within the complex. The first studies on recombination within the B complex identified two genetic regions, a B-F/B-L region containing two different loci, and a B-G region comprised of one locus. The B-F antigens appeared

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homologous to the mammalian Class I antigens, while the B-L antigens appeared homologous to the mammalian Class II antigens. The B-G antigens (class IV), for which no homologues have been found, were initially found expressed only on erythrocytes and were not linked to histocompatibility despite their high polymorphism (Plachy et al., 1992). Later studies, however, have found B-G expression on non-erythroid cells and possible involvement in histocompatibility (Salomonsen et al., 1991).

Cytogenetic methods led to the localization of the B complex to the same chromosome as the nucleolar organizer region (NOR), namely microchromosome 16. The precise mechanism of B complex cytogenetic localization depended on the survivability of birds trisomic for chromosome 16 due to its small size. The observation that birds with three or four nucleoli in their somatic cells were also found by serological methods to express three or four B complex specificities respectively, established the genetic linkage between the NOR and the B system (Bloom and Bacon, 1985; Bloom et al., 1987).

Studies done by Vainio et al. in the 1980s, established the role of B system antigens as restrictors of antigen recognition by T cells and in cell cooperation. Vainio et al. found that cooperation between T and B cells, which is necessary for a successful antibody response to sheep red blood cells (SRBC, a T-dependent antigen), required identity at the MHC between T and B cells, while the response to *Brucella abortus* (BA, a T-independent antigen) did not depend on the requirement for MHC identity between cooperating cells. Furthermore, the same group, using recombinant lines, concluded that the *B-L* region controls antibody formation and germinal center formation, while identity

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at the *B-F* and *B-G* regions is not sufficient for successful T-B cell cooperation (Vainio et al., 1984, reviewed in Plachy et al., 1992).

The discovery of *B-G*, a new region within the MHC unknown in other species, led to an increased interest in that antigen and its potential functions. B-G antigens have been found on erythrocytes, thrombocytes, lymphocytes, and stromal cells of the cecal tonsil, bursa, thymus, and the epithelial cells of the small intestine. The function of B-G has not been elucidated yet. However, it has been associated with some immune functions, such as an "adjuvant effect", which manifests itself as the ability to enhance the production of alloantibodies to other erythrocyte antigens, e.g. B-F, and the generation of rapid, vigorous antibody responses dependent on allelic *B-G* differences between erythrocyte donor and recipient. B-G antigens have also been implicated in the mechanisms responsible for the presence of "natural antibodies", which are antibodies naturally present in the serum of non-immunized animals of various species (Dietert et al., 1991).

Inconsistencies observed between serologically defined *B* complex haplotypes and haplotypes identified by RFLP, suggested the existence of a second, independently-segregating polymorphic MHC-like system in chickens, designated *RfpY* (Briles et al., 1993). Linkage studies and physical mapping of the *B* complex region of chromosome 16, revealed that the *RfpY* system is also found on microchromosome 16 and that it is located between the *B* complex and the region containing the NOR and exhibiting high levels of recombination (Miller et al., 1996). The immune functions of the *RfpY* system are still unclear. However, it has been implicated in the outcome of Rous sarcomas

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(LePage et al., 2000a), as a minor histocompatibility locus (Pharr et al., 1996), and in the outcome of Marek's disease (Pharr et al., 1997; Wakenell et al., 1996).

The chicken MHC demonstrates a strong association with the response to a variety of viral neoplastic. parasitic. bacterial. and autoimmune diseases. Among the neoplastic diseases which show a link to MHC haplotype are: Marek's disease (Briles et al., 1977), Rous sarcoma virus-induced neoplasia (Collins and Briles, 1977), and avian leukosis (Yoo and Sheldon, 1992). Parasitic diseases linked to the *B* complex include eukaryotic infections by members of the genus *Eimeria*, e.g., *Eimeria tenella* (Clare et al., 1985). Bacterial infections, e.g. with *Staphylococcus aureus* and *Pasteurella multocida* (the agent of Fowl cholera) also demonstrate an association with the chicken MHC (Cotter et al., 1992; Lamont et al., 1987). In addition, spontaneous autoimmune thyroiditis in the obese strain of chickens, a model for Hashimoto's disease, also shows an association with the *B* complex (Rose, 1994).

B Complex Structure (Fig. 1). The *B-F/B-L* region, which represents the chicken MHC, has been cloned and sequenced. Compared to mammalian MHC regions, it is very compact. It spans 92 kb, which makes it roughly 20-times smaller than the human MHC. The gene number is also very small compared to mammalian MHCs. There are 19 genes in the chicken *B-F/B-L* region. There are no repetitive elements or repeats in the central portion of the region extending from the class II-β to the class I genes. The average intron size is 200 nucleotides and the average intergenic distances are 30 nucleotides (Kaufman et al., 1999).

There are two class II-β genes and two class I-α genes in addition to nonclassical MHC genes, such as *TAP* (transporter associated with antigen processing) genes, *DM-α*

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and *DM-β* genes (involved in proteolysis of presented antigens), and the *RING3* gene which encodes a nuclear kinase. The single, nonpolymorphic class II-α gene, unlike its mammalian counterpart, is located away from the *B* locus. There is only one class III gene, the complement component, C4, which is located outside of the class I and class II regions. Other class III genes are located in clusters not linked to the MHC. The chicken MHC also contains genes not found in mammalian MHCs, such as one *B-G* gene, which is unique to chickens and encodes a B-G molecule found on B cells, genes encoding C-type animal lectins, and a natural killer receptor gene, *NKr* (Kaufman et al., 1999).

Genes encoding the chicken class IV region, the *B-G* region, are closely linked to the *B-F B-L* region separated from each other by approximately 0.05 cM. (Kaufman et al., 1991). Based on Southern blots using cDNA clones corresponding to B-G molecules, it has been suggested that the *B-G* region is extensive and composed of multiple genes, some of which are nonpolymorphic. That region of the chicken *B* complex has not been sequenced yet (Kaufman and Lamont, 1996).

The major difference between the chicken MHC and mammalian MHCs is the simplicity and compactness of the chicken *B* complex. There are no recombinants found between the class I and class II genes so far. This low level of recombination leads to co-evolution of genes within the MHC such that haplotypes evolve as stable units. This results in co-evolution of genes, such as the *TAP* genes, *NKr* gene, and *DM* genes, together with the classical class II and class I genes, as polymorphic genes, with each combination of alleles at these loci representing a haplotype. In terms of phenotype, this phenomenon manifests itself as a strong association between particular haplotypes and

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resistance and susceptibility to infectious pathogens, the classical example being the association with Marek's disease.

Furthermore, the specificity of chicken MHC molecules is restricted by the fact that of the two polymorphic class I and class II genes, only one is expressed at a high level, leading to a "dominance/recessivity" rather than a "co-dominance" situation. This apparently suicidal strategy of immune recognition defines a "plus/minus" paradigm of immune response in chickens, making them very resistant to some pathogens while susceptible to others. Even though the driving force behind this "minimal essential MHC" strategy has not been clearly defined, it has been proposed that some compensating pressure prevents the up-regulation of MHC expression. One possibility is that Marek's disease, which is an important and virulent disease, imposes a strong selective pressure on chicken MHC specificity and that a simple MHC is necessary to regulate the level of expression of MHC alleles in response to this selective pressure (Kaufman and Salomonsen, 1997).

Minor Histocompatibility Antigens

The development of techniques for the characterization of HLA-associated peptides by Engelhard (1994), led to the identification polymorphic self peptides presented in the context of self MHC molecules, which have an effect on tissue acceptance. These antigens, called minor histocompatibility antigens, are involved in the rejection of tissue grafts even if the donor and recipient are matched at their MHCs but are associated with a less vigorous response than in the case of MHC mismatch. Some examples of minor histocompatibility antigens include the HA-2 antigen, which is a peptide from the non-filament-forming class I myosin family (den Haan et al., 1995), and

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the HY antigen, which is encoded by a gene on the Y chromosome and is therefore only found in men causing rejection of HLA-matched grafts of male tissue by female recipients (Fischer-Lindahl et al., 1991).

Nonclassical Major Histocompatibility Antigens

Nonclassical MHC molecules are involved not only in antigen presentation but also in immunoregulation of both innate and acquired immune responses as well as in nonimmune processes. They are differentiated from classical MHC molecules by their reduced tissue expression and lower level of polymorphism. In humans, some nonclassical MHC antigens include: HLA-E, -F, and -G, MICA and MICB, and CD1. In mice, antigens encoded by the Q, T, and M clusters of the MHC region, are nonclassical MHC proteins (Braud et al., 1999).

HLA-E, which shares homology with the mouse Qa-1 antigen, is involved in the regulation of natural killer (NK) cell activity through binding to NK cell receptors (Braud et al., 1998). HLA-G also appears to be able to regulate NK cell function either directly through binding to NK cell receptors (Verma et al., 1997), or indirectly through up-regulation of HLA-E expression (Liano et al., 1998). The MIC antigens are involved in the activation of γ-δ T cells in the GI epithelium and thus indirectly influence the maintenance of GI epithelium integrity by eliminating infected epithelial cells (Braud et al., 1999). HLA-H, now known as HFE, controls iron metabolism by binding to the transferrin receptor (TfR) on liver and gut cells and modulates the receptor affinity for iron-bound transferrin (Lebron et al., 1998). CD1 antigens are crucial in the process of lipid antigen presentation to T cells and thus significantly influence responses to bacterial infections. To date the role of HLA-F remains uncharacterized (Braud et al., 1999).

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MHC Polymorphism

The capacity for an efficient immune response generated to the immense variety of antigens that an organism encounters during its lifetime depends on the polymorphism of the MHC. With respect to MHC I. a heterozygous individual expresses six major MHC specificities. excluding the contribution of nonclassical MHC I molecules: two *HLA-A* alleles (one from each parent), two *HLA-B* alleles, and two *HLA-C* alleles. The degeneracy of peptide binding, represented as the ability of a single MHC molecule to bind a variety of peptides also contributes to the versatility of MHC-antigen recognition. This versatility of peptide presentation by the MHC is tied in with exquisite specificity. A T cell can discriminate between single amino acid changes in the MHC-bound peptide (Schwartz, 1986) and in the presenting MHC molecule itself (Nathenson et al., 1986).

The fine balance between versatility of peptide binding and specificity on the level of a single amino acid is a function of the interplay between the mechanism for maintaining self-tolerance and processes such as pathogen-driven selection. disassortative mating, and poorly understood reproductive mechanisms leading to the enhanced survival of fetuses which are unlike their mothers (Clarke and Kirby, 1966).

Pathogen-driven selection enhances MHC polymorphism through heterozygote advantage. Selection favors rare haplotypes in order to avoid pathogen evasion of immunity conferred by common MHC specificities (Potts and Slev, 1995). Disassortative mating is a mechanism mediated by the ability of vertebrates to detect MHC haplotype by smell. Studies in mice have demonstrated the propensity of females to mate with MHC-dissimilar males (Potts et al., 1991). Enhanced survival of fetuses heterozygous at the MHC could be mediated by the presence of lethal recessive genes linked to MHC loci.

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For example, the mouse t-complex in which *H-2* is located, contains recessive embryonic lethal genes. Fetuses homozygous for a certain recessive lethal die, while fetuses heterozygous at these loci survive, even if they simply carry different versions of the lethal genes (Bechtol, 1982).

MHC polymorphism not only enhances the survival of the individual, i.e., as a function of the increased number of peptides recognized by heterozygous MHCs, but also promotes the survival of the species as a function of the high number of rare alleles. This is important because pathogens evolve to avoid recognition by common MHC haplotypes. The exact molecular factors which maintain the balance between the high level of MHC polymorphism and the narrow specificity of MHC-peptide-T cell interactions remain to be elucidated.

Other Functions of the MHC

The MHC influences not only immune recognition and susceptibility to infectious and immune diseases, but also individual odors, mating preferences, kin recognition and cooperation, and pregnancy outcome. In fact, the MHC is the first example of a locus which controls complex behavioral patterns serving important functions related to species survival. These behavioral patterns influence mating preferences and kin recognition which result in either kin cooperation, increasing inclusive fitness, or avoidance of matings with kin, to reduce the level of inbreeding. The precise mechanisms controlling mating preferences and kin recognition depend on the ability of MHC differences to influence odors detectable by prospective mates. Studies supporting this hypothesis have been conducted both in mice (Carroll et al., 2002) and humans (Jacob et al., 2002, reviewed in Potts, 2002).

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Accessory Molecules

Antigen presentation and T cell activation are not solely dependent on the interaction between the TCR on T cells and MHC molecules on APCs. These processes are also modulated by accessory molecules. These are membrane molecules which serve the dual purpose of stabilizing the interaction between a T cell and an APC or effector cell by acting as adhesion mediators and transducing signals to the cytoplasm which either enhance or attenuate antigen-driven responses (Aruffo et al., 1992). Based on their function, accessory molecules can be assigned to one or both of the following categories:

(a) surface molecules mediating adhesion to other cells.

(b) signaling molecules, which include immunoglobulin Fc receptors, complement receptors, cytokine receptors, and antigen-receptor associated signaling molecules.

An example of a molecular interaction involved in signal transduction is the one between CD28 on T cells and B7.1 or B7.2 on antigen presenting cells. This interaction provides a signal secondary to antigen recognition in the context of the MHC, necessary for the initiation of naïve T cell responses. A key element of this signal is that together with a stimulus coming through the TCR, it permits production of high levels of IL-2, and thus provides an essential signal for survival and proliferation of T cells, and prevents apoptosis or the induction of anergy, which would occur in the absence of co-stimulation. Alternatively, B7 molecules on APCs could bind a molecule related to CD28, known as CTLA-4, which is a negative regulator of T cell activation (Watts and DeBenedette, 1999). In the presence of large enough numbers of MHC-antigen complexes necessary to initiate adequate TCR signaling, the ensuing immune response

will be influenced by the spatial and temporal distribution of B7-CD28 and B7-CTLA-4 complexes. In the case of low availability of B7 ligands, CTLA-4 signals predominate and inhibit T cell responses, since CTLA-4 has a stronger avidity for CD28 than does B7. In the presence of abundant B7 ligands on APCs, CD28-mediated signals would dominate the immune response and induce T cell proliferation. As a result of T cell activation and IL-2 production, there would be an increase in the synthesis and expression of CTLA-4 molecules and feedback inhibition of T cell proliferation through a rise in the frequency of B7-CTLA-4 interactions (Chambers and Allison, 1997).

Another set of accessory molecules, CD19 and CD21, are part of a multimolecular complex on the surface of B cells, involved in the amplification of signals transduced through the BCR. CD19 has no characterized ligands, while CD21 interacts mainly with C3 fragments of complement. Complement associates with antigen-antibody complexes and thus cross-links the CD19 CD21 complex with the BCR leading to signal amplification and B cell activation and proliferation. In this way, the CD19/CD21 complex provides a link between the innate immune response in the form of complement activation by bacterial polysaccharides, and the acquired immune response in the form of B cell activation (Tsubata, 1999).

Fc receptors mediate a variety of effector functions triggered by cross-linking with the appropriate immunoglobulin. Among these functions are: phagocytosis, antibody dependent cellular cytotoxicity, release of mediators, and enhancement of antigen presentation. CD32, also known as Fc-γRIIb1, is a low affinity Fc receptor for aggregated Ig/immune complexes and is broadly distributed on a variety of immune cells, including B cells and monocytes. In addition to the above-mentioned functions, CD32 can also

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have an inhibitory effect on B cell activation. Cross-linking of the antigen receptor on B cells by antibodies leads to BCR co-aggregation with CD32. This may occur because antibodies that bind the BCR also bind CD32 through the Fc portion of their constant regions. This cross-linking can inhibit signaling through the BCR by sequestration of the antigen receptor away from other signaling molecules necessary for B cell activation (Peaker, 1994).

An example of an adhesion molecule involved in T cell activation is CD2, which binds LFA-3 on APCs and effector cells and stabilizes the binding between T cells and other immune cells. The initial interaction between a T cell and an APC is weak as the T cell scans the surface of the APC for peptides presented in the context of MHC molecules. Recognition of a peptide-MHC complex by the T cell provides a signal which strengthens the affinity of CD2 for its partner on the APC, prolonging the interaction between the two cells and allowing all the signaling events necessary for complete T cell activation to occur (Kuby, 1992).

Lymphocyte activation leads to up-regulated expression of a variety of surface molecules including not only adhesion molecules but also receptors for growth and differentiation factors, necessary for the induction of cell proliferation and differentiation. Among these surface molecules are a variety of receptors for cytokines, such as IL-2 (CD25, CD122, CD132) on T cells, IL-4 (CD124) on B and T cells, and IL-1 (CD121b) on B cells and macrophages. IL-2 is a cytokine produced by T cells which drives the autocrine proliferation and activation of additional T cell clones as well as the growth and differentiation of B cells.In this sense, cytokine receptors "amplify" the immune response

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by recruiting new leukocyte sub-populations and mediating the generation of additional clones of already activated cells.

Of particular interest with respect to B cell activation, is the CD22 accessory molecule, found exclusively on B cells. CD22 has a molecular weight of approximately 135 kD in humans (Tedder et al., 1997) and approximately 150 kD in mice (Torres et al., 1992). There are two serologically distinguishable CD22 alleles in mice (Lajaunias et al., 1999). CD22 appears to be an adhesion molecule since its extracellular domain contains homologous regions to known adhesion molecules. Furthermore, CD22 has been shown to mediate B cell to B cell adhesion in addition to heterotypic adhesion between B cells and various cell types, including erythrocytes, neutrophils, lymphocytes, and monocytes.

CD22 is a sialoadhesin binding to sialic acids and was first associated with the down-modulation of BCR signaling through an increase of the threshold for B cell activation as a result of antigen binding (Stamenkovich et al., 1991). This effect is a function of the ability of CD22 to recruit the protein tyrosine phosphatase, (SHP)-1, which initiates signaling cascades mediating the down-modulation of cell proliferation and antibody production. CD22 has also been implicated in the assembly of complexes for the delivery of positive signals for B cell activation (O'Rourke et al., 1997). This conclusion is based on the observations that CD22 mAb augments B cell proliferation following cross-linking of surface Ig and that CD22 co-precipitates with kinases and phosphatases known to be involved in the activation of B cells. These observations could be the result of sequestration of intracellular inhibitory signaling molecules or the assembly of a separate co-stimulatory complex as a result of CD22 ligation (CD80).

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Based on these seemingly contradictory conclusions about the physiologic function of CD22, it has been suggested that rather than acting as a simple negative or positive regulator of B cell activation. CD22 functions to adjust the signaling threshold of the BCR in response to a variety of conditions related to antigen binding (Tedder et al., 1997). Differential regulation of the expression of CD22 and its ligands determines the conditions under which CD22 is ligated. Interaction between CD22 and its ligands could lead to dissociation of CD22 from the BCR and enhancement of B cell activation by removal of the constitutive inhibitory effect of CD22 on BCR signaling. Since most CD22 ligands are found in secondary lymphoid organs, presentation of antigen at these sites will be associated with CD22 ligation and subsequent lowering of the BCR signaling threshold and increased B cell activation (O'Rourke et al., 1997). In the absence of antigen presentation and adequate levels of CD22 ligands, outside of secondary lymphoid organs. CD22 would localize (SHP)-1 to the cell membrane and thus mediate the down-regulation of spontaneous or inappropriate signaling (Tedder et al., 1997).

CD22 has been shown to bind cells of the sinusoidal epithelium in the bone marrow, thus affecting the migration of B cells to the bone marrow. CD22 also appears to bind CD75, a molecule expressed on mature B cells, as well as IgM, thus influencing humoral immune responses (Tsubata, 1999). In addition to B cell-B cell interactions, CD22, as a possible ligand for CD45 on T cells, has also been implicated in the control of lymphocyte activation. CD45 can have positive or negative effects on the activation of T cells by dephosphorylating intracellular TCR-associated substrates with opposing effects on T cell activation. CD45 function may be regulated by associations with other T cell receptors and is dependent on CD22 binding. It has been proposed that the activation or

inhibition of antigen-receptor signaling could depend on the proportion of CD45 molecules capable of binding CD22 and aggregating with the antigen receptor and accessory molecules (Sgroi et al., 1995).

Despite the abundance of information concerning the structure, ligands, and effects of CD22 based on a variety of *in vitro* experiments and *in vivo* knock-out studies, the precise physiological role of CD22 remains to be elucidated. Based on the characterization of CD22 as a regulatory molecule involved in the setting-up of signaling thresholds for lymphocyte activation, it is possible that inherited polymorphisms in CD22 could have significant effects on susceptibility to infection or predisposition to a variety of autoimmune diseases and other conditions associated with the immune system.

Non-*B* Erythrocyte Alloantigen Systems in the Chicken: Erythrocyte Alloantigen *L (Ea-L)*

There are thirteen erythrocyte alloantigens identified so far in chickens (Briles et al., 1950; Briles, 1962; Gilmour, 1959). These include: *Ea-A, Ea-B, Ea-C, Ea-D, Ea-E, Ea-H, Ea-I, Ea-J, Ea-K, Ea-L, Ea-N, Ea-P,* and *Ea-R*. Erythrocyte alloantigens are by definition cell surface molecules on erythrocytes which differ between individuals within a species. Some of the thirteen alloantigen systems have also been found on cells other than erythrocytes. The majority of the characterized alloantigens are glycosylated, e.g., *Ea-A*. For some of them allelic differences are based on glycosylation heterogeneity, while for others polymorphism is a reflection of protein differences (B-G) (Dietert et al., 1992).

Prior to 1985, erythrocyte alloantigens and their polymorphisms had been identified using alloantibodies, as described on page 18 of this review concerning the *B* complex characterization by Briles et al. (1950). More recently, the investigation of

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alloantigen polymorphisms has been based on the development of probes specific for DNA sequences corresponding to some of the alloantigen groups. These probes have allowed the study of avian alloantigen systems using RFLP and SSCP and have led to the identification of the *RfpY* locus (Chausse et al., 1989).

Most non-*B* alloantigen systems have been functionally characterized to some degree and have been shown to perform immunologically significant roles. Some of them have been associated with minor histocompatibility functions, e.g., *Ea-A, Ea-C* (Schierman and Nordskog, 1965). A large number have been associated with differential antibody response to the T-dependent antigen, sheep red blood cells (SRBC), e.g., *Ea-A, Ea-C, Ea-D, Ea-E*, and *Ea-H*, by observation of allelic shifts following selection for differential antibody response (Dietert et al., 1992). The *A-E*, and *I* systems have been implicated in resistance and susceptibility to *Eimeria tenella*, a protozoan parasite causing cecal coccidiosis in chickens (Dietert et al., 1992). The *K* system has been identified as a vaccinia virus receptor (Gilmour, 1959), while *Ea-R* has been associated with susceptibility to leukosis-sarcoma tumor virus (TVB) (Crittenden et al., 1970).

Erythrocyte alloantigen *L* is a poorly characterized system, discovered independently by Gilmour (1959) and Briles (1962). In 2000, LePage et al. (LePage et al., 2000b) reported an effect of *Ea-L* on the fate of Rous sarcoma-induced tumors. Since then there has been an increased interest in the *L* system, implicating *Ea-L* in a range of immune processes including phagocytosis (Qureshi et al., 2000), and resistance and susceptibility to *Eimeria tenella* (Taylor and Briles, 2000). There has also been progress on the molecular characterization of the Ea-L antigen. Together, these studies have begun

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to paint a clearer picture of the immunological role of *Ea-L* and the mechanism through which it exerts its effect.

Ea-L is a polymorphic alloantigen which has two alleles, L^1 and L^2, segregating at its locus (Gilmour 1959, Briles, 1962). In addition to chicken erythrocytes, chicken leukocytes also appear to express *Ea-L* on their surface (Kopti and Briles, unpublished data). Both the L^1 and L^2 alleles have been determined to encode antigens of approximately 135 kD (Ameiss and Briles., 2000). In a study of blood group polymorphism, following 18 generations of full-sib matings in an outbred line, segregation was maintained at only three loci, among which *Ea-L*, suggesting a possible survival advantage associated with the preservation of heterozygosity (Gilmour, 1958).

In 1979, Etches and Hawes tentatively proposed a linkage between *Ea-L* and the yellow skin locus (*w*), which is located on chromosome 1p. Etches and Hawes did not include *Ea-L* in a map of chromosome 1 which they published in 1979. However, integration of the information given by Etches and Hawes and a recently constructed detailed map of chromosome 1, indirectly points to a possible location of *L* on the small arm of chromosome 1. Etches and Hawes suggested the following order of genes in that region of chromosome 1: *P-O-se-Ea-H-w*, in a direction away from the centromere, with approximately 85 map units between the *P* and *w* loci and 4 map units between the *O* and *P* loci (Etches and Hawes, 1979) [Fig. 2]. In the 2000 consensus linkage map of the chicken genome, the pea-comb (*P*) locus has been mapped at 209 cM from the chromosome 1 p terminus, while the *O* locus has been mapped at 203 cM from the chromosome 1 p terminus (Schmid et al., 2000) [Fig. 3]. This places the *w* locus at approximately 120cM from the p terminus of chromosome 1. Based on this information,

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the search for L could be narrowed down to the 120cM region between the p terminus of chromosome 1 and the w locus.

LePage et al. (2000b) examined segregating combinations of genes encoding eight erythrocyte alloantigen systems in a B^2B^5 and a B^5B^5 MHC background for their effects on Rous sarcomas. Among these alloantigens, $Ea-L$ was the only one which displayed an effect on tumor score, TPI, and mortality in a B^2B^5 genotypic background, and mortality in a B^5B^5 genotypic background. The L^1L^1 genotype was associated with a stronger anti-sarcoma response, manifested as lower tumor score, TPI, and mortality in the B^2B^5 background and lower mortality in the B^5B^5 background than the L^1L^2 genotype.

Taylor and Briles (2000) studied the effects of eight erythrocyte alloantigen systems, including $Ea-L$, on resistance and susceptibility to $E.$ $tenella$-induced cecal coccidiosis in a B^2B^2 and a B^2B^5 background. They found an association only between $Ea-L$ and cecal lesion scores. That effect was evident in a B^2B^2 background but not in a B^2B^5 background. $B^2B^2L^1L^1$ chickens had higher lesion scores than $B^2B^2L^1L^2$ and $B^2B^2L^2L^2$ chickens.

Qureshi et al. (2000) studied the effect of nine erythrocyte alloantigen systems, including $Ea-L$, on the percentage of monocytes exhibiting phagocytosis following in $vitro$ incubation of monocyte monolayers with viable $E.$ $coli$. The L system exhibited a significant effect on monocyte function. In 4wk old birds, the L^1L^1 genotype was associated with a significantly higher percentage of monocytes exhibiting phagocytosis than the L^1L^2 genotype. That effect appeared to be exerted independently of the B system. Later studies by the same group established that $Ea-L$ exerts no effect on nitrite and IL-6 production by macrophages (unpublished data).

Studies before 2000 have suggested a role for *Ea-L* in the humoral branch of the immune system. Scott et al. (1988) found changes in *Ea-L* allele frequencies in chickens divergently selected for bursa size for 21 generations. In the Large Bursa Line, the L^2 allele was present at an average frequency of .02. In the Small Bursa Line, the L^2 allele was present at an average frequency of .54. It is important to note that in the LJD base line, the L^2 allele was not present in any of three generations typed.

In view of the fact that some nonMHC erythrocyte alloantigen systems, among which *Ea-L*, display changes in gene frequencies as a result of selection for bursa size, the authors propose that some of the genes controlling serum IgG levels may be linked to some of these nonMHC systems. *Ea-L* allele frequencies have also been determined in a line selected for primary antibody response to SRBC (Dunnington et al., 1984; Martin et al., 1990). In both the High Line and the Low Line, the L^2 allele was fixed despite the fact that both L^1 and L^2 alleles have been found to segregate in birds of the Cornell randombred stock which formed the base population. However, in neither of the two studies has founder effect been excluded as a possible cause of the observed *Ea-L* frequencies, since the precise genotypes of the sample of Cornell randombred birds used as base population were unknown.

In addition to purely immune functions, *Ea-L* has also been linked to fertility (DeSilva, 1965). DeSilva found that matings in which both parents were homozygous at L, were associated with lower fertility compared to matings in which at least one of the parents was heterozygous. It was suggested that the observed effect is exercised through differential survivability of the fetus.

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Figure 2. Linkage map of chicken chromosome 1. [From: Etches and Hawes, 1973. Can. J. Gen. and Cytol. 15:553-570.]
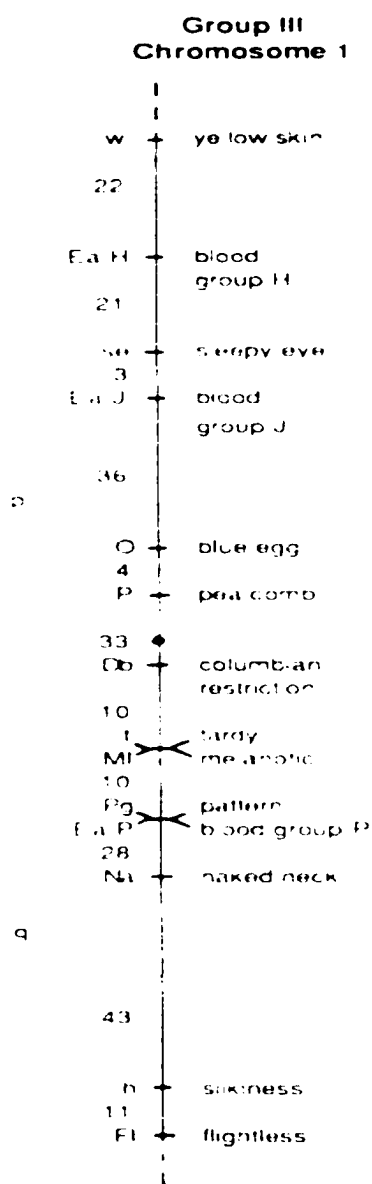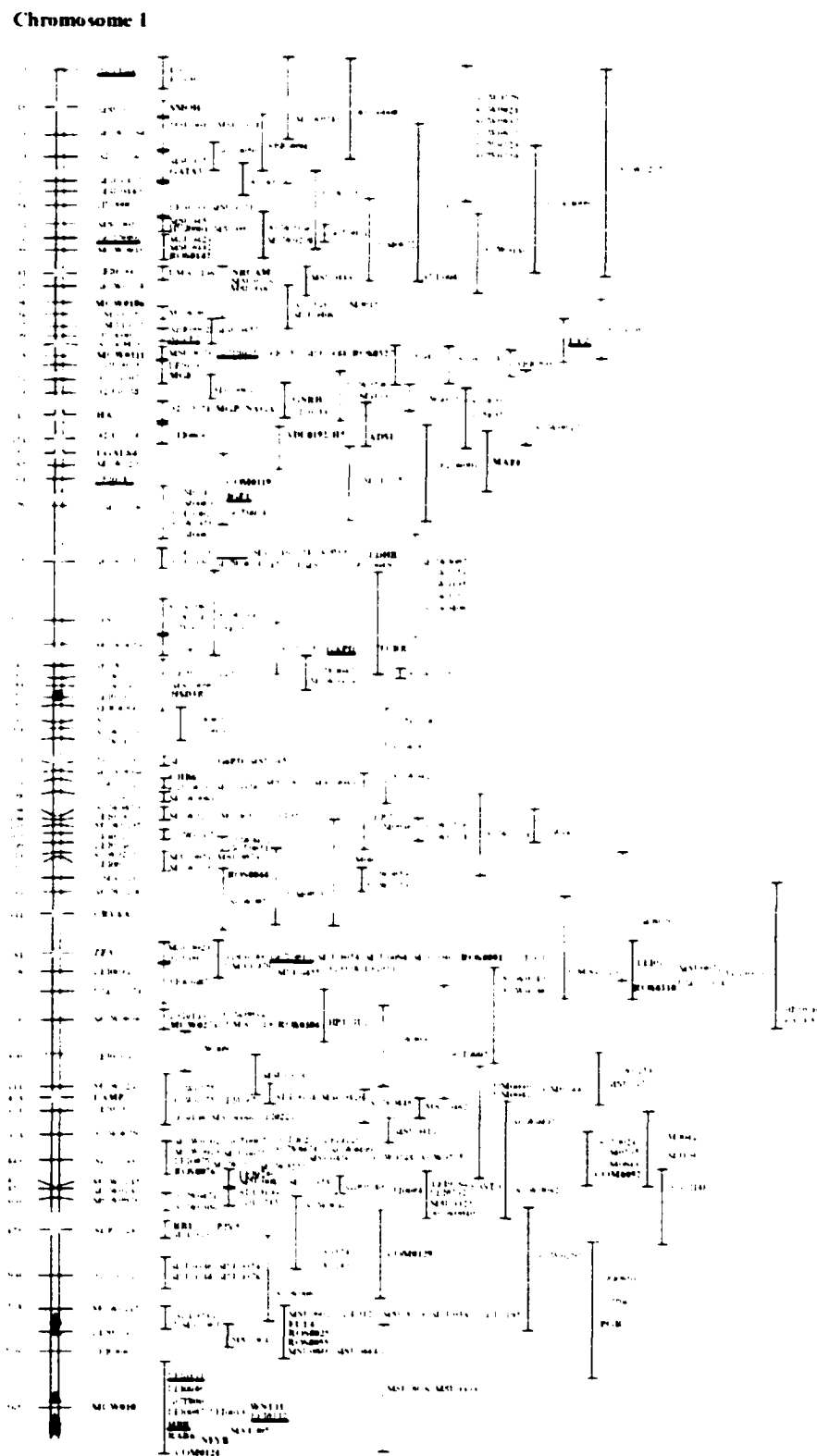

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Figure 3. Year 2000 Linkage map of chicken chromosome 1. The ALVE1 molecular marker represents the *P* and *O* loci mapped to 209 and 203 cM from pter, respectively [From: Schmid M., Nandra I., andD.W. Burt, 2000. First report on chicken genes and chromosomes. Cytogenet. Cell Genet. 90:169-218.]

Chromosome 1

Rous Sarcoma Virus (RSV)

General Features

The Rous sarcoma virus was first discovered in 1911 by Peyton Rous. Rous found a sarcoma localized to the pectoral muscle of a Plymouth Rock hen and passaged it by successive tumor grafts. In the process of identifying the causative agent of this tumor, Rous passed cell-free extracts derived from the tumor through filters which were known to retain bacteria. These cell-free filtrates were shown to also be capable of inducing sarcomas in recipient chickens. Rous identified this mechanism of tumor induction as virus-mediated sarcoma transmission. The study by Rous provides the first evidence of the viral induction of cancer (Rous, 1911).

Initially, avian tumor-virus induction was considered an exception to the rule, rather than a representation of a general principle of tumor induction. Later studies in mammals, however, identified tumor-causing viruses in other organisms. Furthermore, RSV has been shown to be capable of transforming mammalian cells and causing sarcomas in various mammalian species, including hamsters, rats, mice, and monkeys. Currently, it is believed that approximately 20% of human tumors are caused by viruses.

RSV is a member of the family Retroviridae, subfamily Oncoviridae, genus 'Type C oncovirus group'. This classification reflects the oncogenic, retroviral, and structural characteristics of RSV. RSV particles consist of a central core represented by a hexagonally arranged outer shell containing a spiral structure of viral RNA, viral structural proteins (*gag* gene products), and reverse transcriptase (*pol* gene product). A viral envelope, derived from the host cell membrane surrounds the viral particles.

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Beneath the viral envelope is an inner coat characteristically visible in type C viruses. The viral subtype and specificity for host cells is defined by viral glycoproteins (gp37 and gp85), which are encoded in the viral genome (*env* genes) and protrude from the viral envelope (Svoboda, 1986) [Fig. 4].

The process of RSV genome characterization began with the isolation of intact genomic RNA from the virus in 1965 (Robinson et al., 1965) and the demonstration of the double-stranded nature of the RNA molecule in 1968 (Duesberg, 1968). The high resistance of free virus to UV radiation led Rubin and Temin to propose in 1959 that the virus survives inside the host by being integrated in the host genome (Rubin and Temin, 1959).

The unraveling of the mechanics of viral replication began in 1970, when Temin and Mizutani (1970) and Baltimore (1970) found that RNA-dependent DNA polymerase is present in RSV virions and drives the synthesis of proviral DNA from viral genomic RNA. The current picture of viral replication is complicated and involves reverse transcription of one of the two RNA strands, which comprise the viral genome, and synthesis of a DNA strand complementary to the DNA template generated by reverse transcription. This double-stranded DNA molecule enters the nucleus, and circularizes, in the process duplicating its long terminally repeated region (LTR) and creating a series of two LTRs in tandem. In the nucleus, the duplex DNA molecule becomes integrated into the host genome in a manner similar to that described for "insertion elements". The provirus is then transcribed by the host's RNA polymerase II, giving rise to three mRNA species: *gag/pol*, *env*, and *src*. The *gag/pol* mRNA is translated by the host cell's translation apparatus to give rise to the virion core proteins and reverse transcriptase,

Figure 4. Structure of type C oncovirus particles. An RNA molecule is enclosed within a core shell, which is surrounded by an inner coat and an outer coat. The RNA molecule is associated with reverse transcriptase and nucleoproteins. Viral-subtype-characteristic glycoproteins protrude from the outer coat. [Based on Svoboda, 1986]

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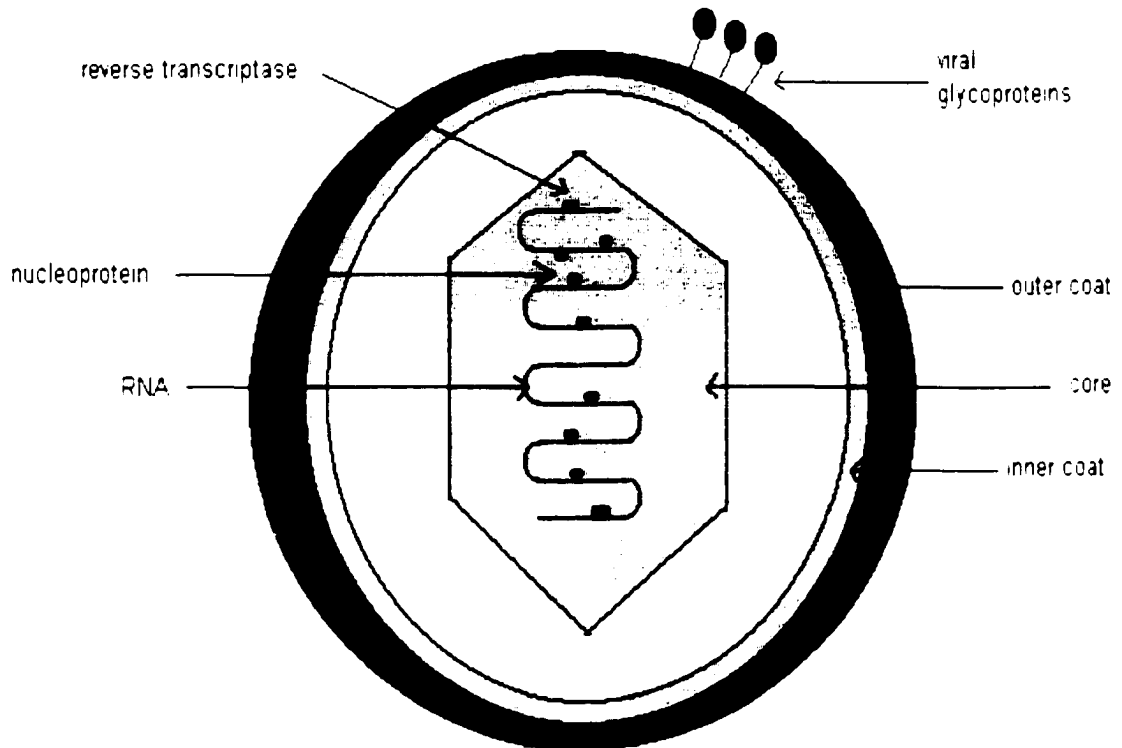
respectively. The *env* mRNA is translated and the resultant protein is processed to produce the gp85 and gp35 viral glycoproteins which are linked by disulfide bonds in the virion. The *src* mRNA encodes the *v-src* viral oncogene, which is responsible for host cell transformation (Svoboda, 1986) [Fig. 5].

In 1976, Stehelin et al., isolated DNA complementary to the RSV genome and hybridized the obtained cDNA with RNA from transformation-detective RSV mutants in order to identify the sequence of the RSV genome responsible for transformation (*v-src*). Using a probe based on this sequence. Stehelin et al. performed hybridization experiments with normal chicken DNA as a template and found sequences (*c-src*) homologous to *v-src* present in a variety of normal chicken cells. This discovery provided the first evidence for the role of cell-derived oncogenes in tumorigenesis and suggested that RSV arose by recombination of a non-transforming RSV ancestor with the cellular *src* proto-oncogene. The process of recombination led to the introduction of mutations into the gene conducive to its oncogenicity (Stehelin et al., 1976).

Since then, *c-src* sequences have been found in all vertebrates where they are highly conserved. In addition, *c-src* product has been identified in primitive multicellular organisms including freshwater sponges (Schartl and Barnekow, 1982). Based on the high level of conservation of the *src* gene, it has been proposed that it may have evolved as an essential mediator of cell-cell contact and differentiation necessary for the organization of all *Metazoa*.

Figure 5. Proviral RSV DNA. On either side of the proviral DNA, there are two LTRs consisting of U3, R, and U5 sequences. The boxes represent coding regions. Gene products of the *gag, pol, env,* and *src* loci are indicated in the boxed areas. [Based on Svoboda, 1986]
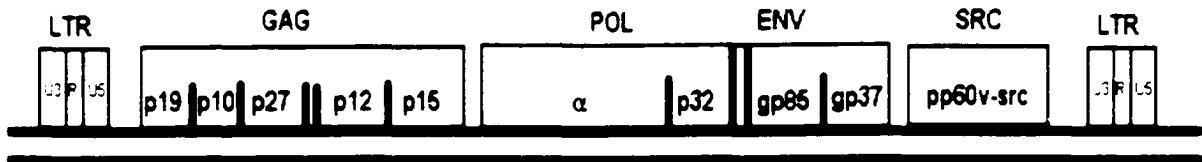
Characterization of the *v-src* oncogene and its cellular counterpart has revealed a lot about the origin of *v-src* and the differences between the viral and the cellular gene conducive to transformation (Fig. 6). *v-src* spans a 1.6-kb region and codes for 526 amino acids. The *c-src* gene is more complicated in that its sequence is interspersed by seven introns not present in *v-src*. It spans between 7 and 8 kb and encodes a 533 amino-acid peptide. *c-src* and *v-src* both contain 12 exons. However, the first exon is not translated but contains a splice acceptor site. *v-src* also incorporates additional sequences, including a small part of an intron upstream from the splice acceptor site of exon 1 and a stretch of 31 bp derived from a region 900 bp downstream from *c-src*, together with 8 nucleotides downstream from the 31-bp insertion derived from the non-transforming RSV ancestor (RAV-2) (Wang and Hanafusa, 1988).

The study of recombinants between different regions of the *v-src* and *c-src* genes has led to the proposition that as few as three amino acid changes between *c-src* and *v-src* can account for their differential transforming potential. The transforming ability of pp60^{v-src} (the product of the *v-src* oncogene) is not a function of the acquisition of new phosphorylation targets but of its increased phosphorylation of normal pp60^{c-src} targets as a result of its constitutive catalytic activity. The activation of pp60^{c-src} represents a shift of phosphorylation from Y527 in the COOH terminus of pp60^{c-src} to Y416 in the kinase domain of the gene (Hanafusa, 1986). In fact, one of the defining differences between pp60^{v-src} and pp60^{c-src} is the truncation and replacement of COOH sequences, such that pp60^{v-src} is missing the Y527 phosphorylation site (Schwartzberg, 1998).

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Figure 6. Comparison between the c-Src and v-Src proteins. Both proteins are myristoylated at Gly2. Positions of the membrane-binding (M), unique (U), SH2, SH3, linker (L), catalytic, and regulatory (R) domains of c-Src are shown. In addition to the carboxy-terminus substitutions in v-Src, there are additional amino-acid substitutions along the length of the protein, shown as black dots. [Based on Wang and Hanafusa, 1988]

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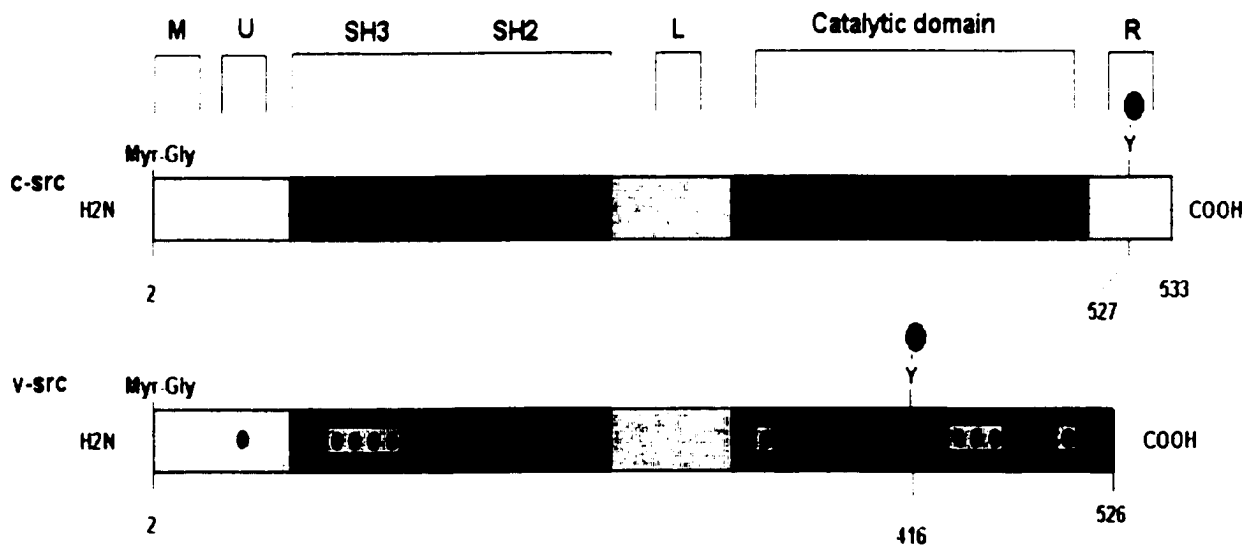
In the inactive state, Y527 engages the SH2 domain of the kinase and as a result, Src adopts a "closed" conformation, such that the SH2 and SH3 domains responsible for substrate recognition are inaccessible to other proteins. In *v-src* encoded Src, the inhibitory Y527 is missing from the COOH terminus of the protein, the SH2 and SH3 domains are exposed and the protein adopts an "open" conformation, such that it can interact with its substrates. Furthermore, the "open" conformation is stabilized by autophosphorylation of Y416 in the kinase domain of the protein. As a result *v-src* encoded Src kinase is active and capable of constitutively phosphorylating its substrates (Brown and Cooper, 1996) [Fig. 7].

The effort to identify the substrates of Src has led to the compilation of a long list of proteins with diverse functions (Table I). These proteins can be classified into two general groups: proteins implicated in mitogenic signaling pathways, e.g., RasGAP and PLC-γ, and proteins involved in cytoskeletal organization, cell-substrate adhesion, cell-cell adhesion, and cell-cell communication, e.g., connexin, caveolin, vinculin, cortactin. This duality of substrates suggests a pleiotropic role for Src in growth control on the one hand, and modulation of the cytoskeleton, on the other. These effects make Src an especially efficient oncogene since it can transform both by up-regulating mitogenic stimuli and altering the cytoskeleton and adhesion properties of the cell.

It has been shown that Src, which normally resides in perinuclear, plasma, and nuclear membranes, upon mutational activation concentrates in focal adhesions where actin filaments associate with membrane proteins and thus establish contact with the extracellular matrix. This relocation of Src upon activation is essential for its transforming potential (Brown and Cooper, 1996). In RSV-transformed cells, Src

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localizes to adhesion plaques where stress fibers anchor the cell to the surface of the culture vessel. This interaction of Src with stress fibers could potentially mediate the dissociation of these fibers and the reorganization of cell morphology resulting in the typical rounded shape of RSV-transformed fibroblasts (Svoboda, 1986).

Because of the wealth of information that has been compiled about the Rous sarcoma virus and the *v-src* oncogene, RSV has become one of the principal models for the study of cell transformation. Based on studies of the Rous sarcoma virus and its oncogene, a model of transformation has begun to emerge that involves the alteration of both cytoskeletal and metabolic components of the normal cell. As a result of these alterations, the cell becomes permanently transferred into an abnormal steady-state program. While in the normal cell the steady state is defined by the cell's interactions with its environment, in the transformed state, the cell machinery is able to escape the regulatory mechanisms that operate in the normal cell. This leads to an altered, "transformed" phenotype characterized by morphological and metabolic changes affecting cell shape, the cytoskeletal structure, the mobility of surface receptors, cell-cell adhesion and adhesion with the extracellular matrix, aerobic glycolysis and glucose transport, to mention a few (Singer et al., 1980).

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Figure 7. Two states of Src: a) an "open", active state, and b) a "closed", inactive state. In the "closed" state, the SH2 and SH3 domains are inaccessible to other proteins due to being engaged with the phosphorylated C-terminal Y527. In the "open" state, the SH2 and SH3 domains are accessible. The "open" state is stabilized by phosphorylation at Y416. [Based on Brown and Cooper, 1996]

a) Src open conformation
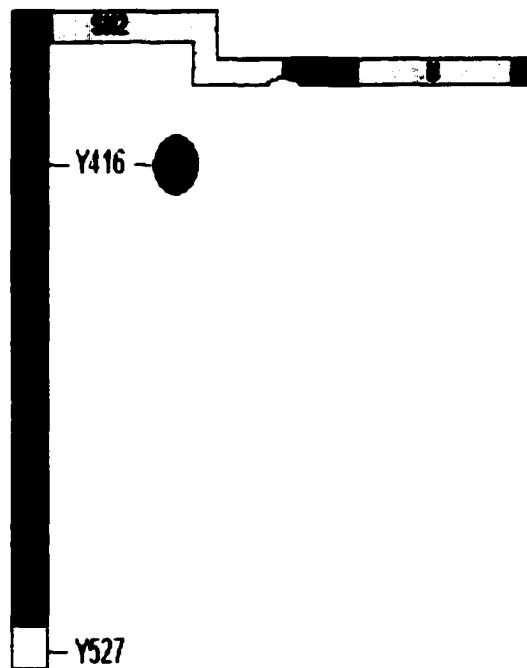
b) Src closed conformation
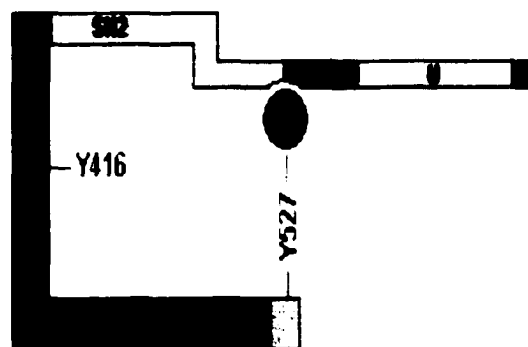
Table I. Src substrates. [Source: Brown T.M. and J.A. Cooper, 1996. Regulation, substrates and functions of src. Biochim. Biophys. Acta 1287:121-149].

p85 PI3 kinase	non-catalytic subunit of PI3 kinase
RasGAP	GTPase activator for Ras
SHPTP2/Syp	PTP
Shc	binds Grb2
PLCγ	PI-specific phospholipase C
p62	unknown
p190	GTPase activator for Rho
tensin	actin-binding, integrin-signaling
vinculin	actin-binding, integrin-signaling
cortactin	cortical actin binding
talin	actin and integrin binding
paxillin	integrin signaling
AFAP110	actin filament associated
FAK	tyrosine kinase, integrin binding
β1 integrin	cell-substrate adhesion and signaling
p130^{CAS}	integrin signaling
p120^{CAS}	catenin, cell-cell adhesion
β-catenin	cadherin binding, cell-cell adhesion
plakoglobin	cell-cell adhesion
connexin 43	gap junctions
Sam68	RNA binding
caveolin	caveolar structure and function
calpactin I	Ca^{2+}/phospholipid binding

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Immune Response to RSV

The first evidence that the fate of RSV-induced tumors may be under host genetic control came from selection experiments. Greenwood et al. (1948) identified birds that had a regressive response to subcutaneous inoculation with RSV, and made selective matings for two generations to produce an offspring characterized by high levels of regression. Gyles et al (1968) differentiated between two types of response to RSV inoculation: resistance, defined as the absence of transformation of normal cells to malignancy, and regression, defined as the reduction in tumor size and complete disappearance of the tumor over time. Studies by Gyles and Brown (1971) suggested, based on the rate of increase in regression as a result of selection, that the response to Rous sarcomas is controlled by a single pair of alleles or a small number of genes with low penetrance. Resistance to the formation of Rous sarcomas was ascribed to a single pair of alleles with resistance being recessive to susceptibility (Crittenden et al., 1964). Currently, it is believed that resistance to RSV transformation is caused by the absence of RSV-specific receptors on the cells of resistant chickens.

In 1977, Collins et al. studied the fate of RSV-induced tumors in an F_2 population segregating at three alloantigen loci: B, D, and I. The authors found that among the F2 B^2B^2, B^2B^5, and B^5B^5 segregants, the mortality by 70 days post-inoculation was 5, 26, and 93% respectively. In addition, the incidence of metastatic lesions from Rous sarcomas was significantly associated with B genotype. Based on these findings, Collins suggested that the fate of Rous sarcomas is controlled by genes within or linked to the B complex. These results, together with previous studies (Cotter et al., 1976a; Cotter et al., 1976b)

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which indicated a cell-mediated immune control of the fate of Rous sarcomas, led to the following possible models:

1. The *B*-locus effect is a function of a similarity between a tumor-associated antigen and the B$_5$ antigen, such that self-tolerance leads to lack of tumor regression;

2. a virus-modified B antigen acts as a tumor antigen, so that different B antigens vary in antigenicity;

3. a B antigen is directly involved in antigen recognition by T lymphocytes.

So far support has been found for all three models and will be discussed later in this section.

The region of the *B* complex responsible for tumor regression was further narrowed down by the use of *B* complex recombinant lines. Auclair et al. (1995) found significant differences in tumor outcome as a function of *B-L B-F* haplotype but not *B-G* haplotype. Since the linkage between the *B-F* and *B-L* regions is very tight, differences between tumor outcome as a function of the *B-F* region separately from the *B-L* region have not been reported.

Studies of the growth patterns of tumors induced by various strains of avian sarcoma virus in partially congenic inbred lines of chickens homozygous for different MHC haplotypes led to the observation that the ability to regress tumors behaves as a dominant trait controlled by MHC-linked genes (Auclair et al., 1995). In a similar study, McBride et al. (1981) showed that the ability to regress tumors is not a function of virus subgroup and therefore excluded determinants on viral coat proteins, e.g., gp85, as key players in MHC-mediated tumor regression. However, variability in tumor growth was

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observed with respect to viral strain. McBride et al. suggested that the structural polymorphism and viral-strain-specific immunogenicity of the src product, $p60^{src}$, makes it a plausible candidate for the target of MHC-mediated anti-Rous-sarcoma immune responses.

Following the identification of the B complex as the region most strongly influencing the fate of Rous sarcomas, studies of lines identical at the MHC and differing in their background genomes or lines of identical genetic backgrounds and different MHCs, suggested the contribution of genes other than the MHC in the determination of tumor fate. In a classic study, Brown et al. (1984) compared tumor fate in hosts of identical MHC genotypes and differing with respect to the line from which they derived their background genomes. The birds whose background genes were derived from one inbred line regressed their tumors, while the birds whose background genes were derived from another inbred line died from progressive sarcoma growth. These differences were ascribed to the influence of nonMHC, background genes on tumor fate.

Gilmour et al. (1976, 1983), implicated specific T cell markers, Ly-4 and Th-1, and the B cell marker, Bu-1, as nonMHC modulators of Rous sarcoma outcome. Gilmour studied tumor fate in birds identical at the MHC but segregating at the Ly-4 and Th-1 loci in the first study, and the Ly-4 and Bu-1 loci in the later study. Gilmour et al. determined that the interaction between particular genotypes at these loci affected tumor growth. Namely, they found that when the two loci are homozygous and in repulsion with respect to each other, high levels of regression were evident in identical MHC backgrounds.

In a study of Rous sarcoma metastasis in chickens, Collins et al. (1985a) also found evidence for the influence of nonMHC genetic factors on tumor metastasis. In $B^{5}B^{5}$

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(progressor) hosts from a $(6_1 \times 15_1)F_2$ population, the incidence of metastasis was 60%, while in $B^s B^s$ hosts from a White Leghorn-New Hampshire F_2 population, the incidence of metastasis was only 31%. It appears that differences between the genetic backgrounds of the two lines mediated these differential effects.

Our understanding of the immune mechanisms of Rous sarcoma regression is rudimentary at this point. Both cell-mediated and humoral factors have been implicated in the response to Rous sarcomas. Radzichovskaja (1968) found that the regression of RSV-induced tumors is associated with the production of antibodies capable of agglutinating homologous tumor cells. Selection for ability to regress Rous sarcomas was accompanied by the generation of significantly higher antibody titers and higher levels of serum immunoglobulin (Carte et al., 1972). McBride et al. (1978) provided further evidence for the role of humoral immunity in tumor regression by showing that inbred $B^2 B^2$ regressor birds were rendered susceptible to tumor growth as a result of bursectomy.

More recently, two studies (Gyles et al., 1986 ; Haddad et al., 1994) have investigated differences in antibody titers to SRBC, and *Salmonella pullorum* (SP) in two lines divergently selected for RSV-induced tumor outcome: a Regressor line, which typically regresses sarcomas, and a Progressor line, which develops fatal tumors. In both studies, the Regressor birds were found to have an earlier humoral immune response to SRBC. The study by Haddad found that the Regressor line had an overall higher titer to SP throughout the trial than the Progressor birds, while the study by Gyles found no such differences. This observation by Gyles was ascribed to competition between antigens, since the same birds were inoculated with five antigens within a short span of time.

In studies examining the contribution of cellular immunity to tumor regression, Sjogren and Jonsson (1970) demonstrated that thymus cells from tumor-bearing chickens reacted specifically with tumor cells *in vitro*. Comparison of the levels of residual thymus tissue with degree of tumor regression in thymectomized birds injected with RSV showed a positive correlation between amounts of residual thymus tissue and regression (Cotter et al., 1976a). In addition, migration inhibition of peripheral lymphocyte populations by soluble tumor extract was greater in regressor chickens than in chickens with progressing tumors (Cotter et al., 1976b).

Histological examination of Rous sarcomas (Powell et al., 1987) led to the intriguing proposition that tumor cells from regressor birds were positive for B-L (MHC II) antigens, while tumor cells from progressor birds were negative for these antigens. B-L antigen expression is normally restricted only to lymphoid cells and professional antigen presenting cells, such as macrophages and dendritic cells. This finding implicated MHC-II-mediated immunity as a significant factor in the definition of anti-tumor responses. Such aberrant expression of MHC II antigens has been observed in other species in association with autoimmune and malignant diseases, as well as parasitic infections. These antigens potentially influence the immunogenicity of the tumor and through this mechanism enhance the "visibility" of the tumor for immune attack and lead to tumor regression. In addition, the same study found increased infiltration of tumors from regressor birds by lymphocytes, particularly activated T cells, providing further support for the importance of T cell mediated immunity in tumor fate.

The possibility of cross-reactivity between B_5 MHC antigens and tumor-associated antigens as a possible factor influencing tumor fate has also been investigated.

B^2B^2 chickens made partially tolerant to the B_5 antigen demonstrated a higher incidence of tumor progression than untreated B^2B^2 birds (Heinzelmann et al., 1981b). This finding was further supported by *in vitro* studies, in which lymphocytes from B^2B^2 birds bearing RSV-induced tumors lysed targets of uninfected B^5B^5 chicken-embryo fibroblasts (CEF) as well as RSV-infected B^2B^2 and B^5B^5 chicken embryo fibroblasts. Serological examination of CEFs showed that they expressed B-F antigens and that B^2B^2 RSV-infected CEFs were capable of absorbing out antibodies from B^5B^5 alloantisera, giving further support to the idea that such cross-reactivity exists (Heinzelmann et al., 1981a).

Results contradicting the proposition of cross-reactivity between MHC antigens and tumor-associated antigens have also been reported (Plachy and Spatenkova, 1982). This study, however, used different B haplotypes and virus subgroups.

RSV oncogenesis reflects not only the contribution of clonal expansion of tumor cells but also the additional influence of viral recruitment on oncogene dissemination. This model thus diverges from the majority of naturally occurring neoplasms in which clonal expansion is the central contributor to neoplasm etiology. Studies in the late 1980's and the 1990's, however, demonstrated that injection of chickens with replication-defective retroviral vectors, which lacked both viral replication and viral structural and envelope sequences and contained only the *v-src* sequence of RSV, gave rise to aggressive clonal tumors and metastasis (Stoker and Sieweke, 1989).

In 1991, Halpern et al. studied the factors influencing *v-src*-induced tumor fate and found that the pattern of growth (regression vs. progression) of the tumors was modulated by the line of chickens inoculated. This finding suggested that *v-src*-induced sarcomas are under host genetic control.

Taylor et al. (1992, 1994) demonstrated that both the growth pattern of v-src-induced sarcomas and the spread of metastasis resulting from these tumors were controlled by the B complex. Taylor suggested a possible mechanism underlying the B complex control of v-src tumor growth, which involved a differential immune response to tumor-specific antigen(s). Furthermore, Taylor et al. observed the same segregation of B haplotypes with respect to tumor regression vs. progression of v-src DNA-induced tumors as had been observed with respect to tumor regression vs. progression of RSV-induced tumors. The B^2 haplotype is associated with tumor regression, while the B^5 haplotype is associated with tumor progression. This led the authors to propose that B complex control of RSV-induced tumor fate is strongly defined by the response to some v-src-determined function, possibly the immune recognition of a tumor-specific antigen.

Such antigen has been identified as a result of the transformation of hamster fibroblasts by an env strain of RSV. It is a virus-induced nonvirion antigen (VCSA), which is specific for transformation and whose expression is controlled by the src gene. This antigen is comprised of three distinct antigenic specificities: one expressed on all RSV-transformed fibroblasts, one cross-reacting with an antigen found on untransformed avian fibroblasts but not mammalian fibroblasts, and one species-specific and found only on RSV-transformed hamster fibroblasts. Based on the identification of these three specificities, it was suggested that the expression of the VCSA antigen was the result of an interaction between $pp60^{src}$ with host cell genes (Prat et al., 1980).

The investigation of the immune mechanisms controlling RSV tumor regression is still in progress. It is possible that genes other than the MHC control v-src DNA-induced tumor fate. The RSV and v-src systems are also an excellent model for the study of the

distinctions between neoplastic disease resulting from viral infection and neoplastic disease resulting from the introduction of mutations of nonviral origin. Identification of the genetic factors contributing to tumor immunity has a therapeutic potential and could lead to a better understanding of the immune system.

Sheep Red Blood Cells (SRBC)

General Features

Sheep erythrocytes are a complex, multideterminant natural antigen, which provokes a T cell dependent immune response. T lymphocytes sensitized to heterologous erythrocytes bind complex antigenic structures on these erythrocytes and form rosettes-clusters of erythrocytes surrounding a T lymphocyte. B cells also form rosettes upon exposure to heterologous erythrocytes, which are more complex and consist of more than one B cell.

Human T lymphocytes possess a specific receptor for sheep erythrocytes, CD2 (also known as T11), which binds T11TS, a glycoprotein found on sheep erythrocytes and involved in the formation of rosettes. Evidence for the specificity of the reactions of rodent and avian lymphocytes to SRBC comes from the fact that active immunization with the antigen results in a dramatic increase in the number of antigen-binding cells reactive with the antigen but no increase in the number of cells binding non-cross-reacting antigen (Hunig et al., 1987).

Immune Response

The immune response to SRBC in the chicken has been demonstrated to be under quantitative host genetic control. Siegel and Gross (1980) subjected the same population

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of birds to divergent selection for antibody response to SRBC and obtained a positive response. Van der Zijpp et al. (1983) demonstrated additive genetic effects on antibody response to SRBC and obtained heritability estimates of 0.39 for primary antibody titers and 0.28 for secondary antibody titers. Dunnington et al. (1984) concluded that the B complex exerts an effect on antibody response to SRBC as a result of observed allele frequency differences at the B complex between lines divergently selected for antibody titer. In a later study, Dunnington et al. (1989), found a significant effect of background genes as well as the MHC on antibody titer to SRBC in lines divergently selected for the trait. In the high-titer line, chickens heterozygous for the MHC had higher titers than either of the homozygotes, while in the low line, the $B^{21}B^{21}$ birds had higher titers than the other two genotypes, suggesting contribution from background genes. Later, Dunnington et al. (1996) confirmed the strong but not absolute association between antibody response and MHC haplotype. In a separate study, Martin et al. (1990) found that following 13 generations of divergent selection for SRBC titer, the high line had a B^{21} allele frequency of 99%, while the low line had a B^{13} allele frequency of 98%.

Interestingly, the B^{21} haplotype is a classic example of the effect of MHC haplotype on viral diseases in the chicken, namely Marek's disease, which is a disease caused by an oncogenic herpesvirus and characterized by lesions of the nerve endings. The B^{21} haplotype is associated with increased resistance to the disease, compared to the B^{19} haplotype. With respect to the B^2 and B^5 haplotypes, associated with regression and progression of RSV-induced tumors, respectively, Dix and Taylor (1996) found that in two congenic lines, B^5B^5 birds produced higher titers than B^2B^2 birds. The authors suggested differences in macrophage function (antigen persistence at the macrophage

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surface vs. macrophage tumoricidal activity) as a possible explanation for the inverse relationship between haplotypes with respect to RSV-induced tumor fate on the one hand and SRBC titer, on the other. Similar studies correlating antibody titer in birds selected for SRBC antibody response on the one hand, and a variety of diseases, on the other, have led to the general proposition that lines selected for high antibody response exhibit greater resistance to viral and parasitic infections (Newcastle disease virus, splenomegalia virus, MDV, and *Eimeria tenella*) and greater susceptibility to bacterial infections (*E. coli* and *S. aureus*) (Yang et al., 2000).

Theories about the effect of selection on antibody response to SRBC focus on contributions not only of B cells but also of macrophages and T lymphocytes in the fine-tuning of the response. In mice, Biozzi et al. (1984) attributed differences in antibody production between lines selected for high or low antibody titer to SRBC to differences in the multiplication rate of B lymphocytes as well as differences in antigen handling by macrophages. Macrophages from the low responder line were found to have a higher metabolic rate. It was hypothesized that the higher catabolic rate of macrophages could leave less SRBC to trigger a B cell response and thus explain the reduced effectiveness of the antibody response in the low responder line. Furthermore, Biozzi et al. suggested that the antibody response to SRBC is qualitatively affected by antigen dose. In the low responder line, a larger threshold dose was necessary to trigger an antibody response due to the high efficiency of macrophages at clearing the antigen. Martin et al. (1990) also suggest the existence of a threshold for triggering an efficient antibody response to SRBC in chickens as a result of the observation that the frequency of responders and nonresponders changes at lower or higher doses of antigen.

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The contribution of T cells to antibody response to SRBC has also been investigated. In a line of chickens selected for high titer to SRBC, mitogen response and cell-cycle activity of T lymphocytes were significantly higher than in the low responder line. These results suggested that selection for antibody response to SRBC indirectly altered T-cell activity and, consequently, that one of the factors influencing antibody response to T-dependent antigens is the level of T-cell activation following challenge (Scott et al., 1991). A later study (Kreukniet et al., 1994) contradicted these observations. In lines divergently selected for antibody response to SRBC, T lymphocytes from the low responder line were found to have a higher mitogen response than T cells from the high responder. The two studies, however, used different lines of birds, selected for antibody titer to different doses of antigen, which as stated previously can result in qualitative differences in the immune response.

Histological and flow cytometry examination of immune organ constitution and lymphocyte subpopulations of lines divergently selected for antibody response to SRBC revealed that the high responder line has relatively more B cells and the splenic structure of birds from that line is better organized for mounting an efficient T-dependent immune response. The percentage of circulating T lymphocytes was found to be higher in the low responder line. However, soon after immunization, T lymphocytes are trapped in lymphoid organs to aid in the elimination of antigen. The high responder line appeared to have higher numbers of CD4⁺ T cells in secondary lymphoid organs following immunization, while the low responder line had higher numbers of CD8⁺ T cells in secondary lymphoid organs. The higher numbers of CD4⁺ T cells and B lymphocytes in the spleens of the high responders apparently lead to an enhanced capacity to deal with a

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T-dependent antigen. Presumably, the high numbers of CD8$^+$ T cells in the spleens of low responders constitute a compensatory mechanism for dealing with the antigen through a cytotoxic or suppressor function (Kreukniet et al., 1996).

Studies of antibody response to SRBC evaluate not only the antibody titer to primary immunization but also to secondary immunization with the antigen. Furthermore, conclusions are based not only on measurements of total antibody titers but also of 2-Mercaptoethanol (ME)-resistant (IgG) and –susceptible (mainly IgM) antibody titers, in order to evaluate the contribution of both isotypes. IgG constitutes the major portion of the antibody response and is capable of neutralizing viral infectivity, contributing to bacterial immunity, opsonizing antigen, and mediating interactions with other immune components, e.g., NK cells. IgM, on the other hand, is the primary antibody involved in hemagglutination reactions, and activation of complement. While the major antibody participating in a primary antibody response is IgM, secondary antibody responses are primarily comprised of IgG antibodies. For this reason, distinctions between primary and secondary, IgG and IgM antibody titers, contribute to a better understanding of the kinetics and constitution of an antibody response, rather than simply of its magnitude.

In a study of birds divergently selected for primary antibody response to SRBC, the differences between lines with respect to primary response were not evident following secondary and tertiary injections. This was due to the presence of immunological memory, defined as a more rapid and vigorous response following a second encounter with an antigen, only in the low line but not in the high line, and suggested that primary and secondary responses are under different genetic controls. Furthermore, the authors proposed that a high initial response in the high line may preclude resources needed for a

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memory response following secondary and tertiary injections. These results, however, were influenced by age of primary injection, and thus the conclusions from them could not be generalized (Boa-Amponsem et al., 1999).

An investigation into the kinetics of IgG and IgM responses to SRBC (Martin et al., 1989) found a difference between the high and low lines in the antibody composition of secondary responses. In the low line, total, ME-sensitive, and ME-resistant titers peaked and persisted following the same pattern with respect to each other. In the high line, total and ME-resistant titers peaked and persisted at higher levels, whereas the ME-sensitive titers declined. Primary antibody titers also differed between the two lines. While in the low line, amounts of ME-sensitive antibody persisted at low levels, in the high line they declined with time. Thus, in the low line, both primary and secondary responses were predominantly IgM, while in the high line secondary responses consisted mainly of IgG. Therefore, the authors conclude that although selection would lead to higher titers both of IgG and IgM antibodies, this change is not symmetrical and is influenced by the time and efficiency of isotype switching to IgG.

Brucella abortus *(B.A)*

General Features

Brucella abortus (BA) is a Gram-negative non-spore-forming coccobacillus. BA possesses a cell-wall characteristic of Gram-negative bacteria but distinguished by the unusual nature of the noncovalent bonding forces that bridge the lipopolysaccharide (LPS), the outer membrane proteins, and the cell-wall peptidoglycans (Baldwin and Winter, 1994).

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LPS on the surface of BA is characterized as a T-independent antigen based on its ability to activate B lymphocytes and induce the production of antibodies without a contribution from T cells. When conjugated to trinitrophenyl (TNP) in a standard assay for T dependence, BA elicited anti-TNP antibodies both in normal mice and in athymic and neonatal mice. Athymic mice are deficient in T cells, while neonatal mice are unable to respond to antigens classified as T-independent type 2 antigens. These observations led to the conclusion that BA is a type 1 T-independent antigen carrier. The same conclusions were made in the human system (Golding et al., 1980).

Immune Response

The immunogenic antigen of BA is the O-polysaccharide side chain of the LPS, which is a homopolymer of 4,6-dideoxy-4-formamido-alpha-D-mannopyranosyl residues. Winter et al. were able to show that protective antibodies are specific to the O-polysaccharide of LPS in mice and could prevent infection after a sufficiently low challenge dose (Winter et al., 1988). Furthermore, it has been demonstrated that CD4⁺ and CD8⁺ T cells are also involved in the establishment of protective immunity against BA through passive transfer and depletion experiments (Baldwin and Winter, 1994).

Macrophages are known to play a central role in the progression of a BA infection. BA bacteria infect phagocytes and survive inside them by inhibiting phagosome-lysosome fusion, thus altering the process of phagosome maturation. In the course of an infection, phagocytes transport the bacteria to lymph nodes. *En route* some infected macrophages lyse and release bacteria into the circulation. As a result free bacteria are carried into the liver, spleen, bone marrow, and kidneys where they cause lesions. In ruminants, BA infects the placenta and fetus of gestating mothers and causes

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abortions. In humans, the classic symptoms include severe fever, chills, fatigue, and weight loss (Wright, 1987). It has been demonstrated that macrophages treated with IFN-γ inhibit bacterial replication *in vitro*. *In vivo*, administration of antibody to IFN-γ worsens the infection. These findings suggest that BA infection is controlled by macrophages which have been activated by IFN-γ and other cytokines known to be produced by activated T_h cells (Hovver and Friedhandler, 2001). Furthermore, it has been shown that efficient phagocytosis of BA bacteria is dependent on opsonization by antibody (Baldwin and Winter, 1994).

These findings have begun to paint a clearer picture of the anti-BA immune response, and have led to the re-classification of BA as a partially-T-independent carrier. It appears that immunity to BA involves the full gamut of immune cells, including macrophages, antigen-specific $CD4^+$ and $CD8^+$ T cells, and B cells. Pathogen-specific antigenic patterns are recognized by Toll-like receptors on APCs, which activate these cells and lead to phagocytosis of bacteria. Alternatively, opsonized bacteria are recognized by means of Fc receptors. Internalized bacteria are killed and peptides generated from them are presented in the context of MHC molecules. In that context, bacterial antigenic patterns activate T_c cells or T_h cells. T_h cells release cytokines, which activate B cells to secrete antibodies or phagocytic cells to kill internalized bacteria. The distinction between the establishment of chronic infection and immunity rests on the balance between the ability of BA to survive inside macrophages by inhibiting phagosome maturation or apoptosis of infected cells and the capacity of the immune system to activate macrophages to kill internalized bacteria (Golding et al., 2001).

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The T-independence of BA is a function of its ability to induce antibody production in T-cell deficient hosts. The mechanism of behind T-independence, however, is not clear. It has been proposed that polymeric antigens are T-independent since they are capable of cross-linking antigen-binding receptors on B cells, while polysaccharide antigens are T-independent since they fail to bind MHC molecules on APCs. Originally, it was believed that LPS, being a polysaccharide, fails to bind the MHC II groove and thus fails to activate T cells. However, there has been overwhelming evidence that bacterial polysaccharides, BA smooth LPS, and isolated O-chain in particular, can bind MHC II molecules on B and T lymphocytes and activate mouse and human T cells in an antigen-presenting-cell-dependent manner (Forestier et al., 1999).

The partial T-dependence of BA antigen has also been demonstrated in chickens (Trout et al., 1996). Following BA injection into chickens, peripheral blood lymphocytes and spleen lymphocytes were isolated and characterized. The study found redistribution of CD4$^+$ T cells from the blood to the spleen, indicating sequestration in secondary lymphoid organs, and reduced levels of CD8$^+$ T cells both in the blood and in the spleen, suggesting reduction in the numbers of suppressor T lymphocytes. Interestingly, B lymphocytes in the blood and the spleen remained unchanged.

The majority of the studies involving BA in chickens have been based on the examination of humoral responses to BA following selection for different immune parameters. In 1992, Dunnington et al., utilizing a line of chickens selected for antibody response to SRBC, found that selection for SRBC also results in divergent antibody responses to BA. Furthermore, Dunnington et al. proposed that the antibody response to BA was influenced both by MHC haplotype and genetic background. In a subsequent

study, Scott et al. (1994) also concluded that birds selected for SRBC exhibited divergent anti-BA titers and that both primary total and ME-resistant titers in the high line were higher than the titers in the low line, suggesting that similar forces control antibody response to SRBC and BA.

In birds selected for multitrait immunocompetence (antibody levels, macrophage function, and cell-mediated immunity), divergent antibody responses to BA were only evident with respect to secondary immunization, while divergent antibody responses to SRBC were present with respect to both primary and secondary immunization, suggesting a difference related to T cell function (Nelson et al., 1995). Selection for high serum IgM and high serum IgG concentrations also resulted in divergent anti-BA titers. The high IgM and high IgG lines had significantly higher titers than the low lines. These findings support the hypothesis that selection for serum immunoglobulin levels leads to changes in the overall multiplication and differentiation of B cells capable of initiating antigen-specific responses. Therefore, factors influencing serum Ig levels would also influence titers against BA.

The effect of MHC haplotype on immune response to BA was studied in lines congenic for the MHC (Dix and Taylor, 1996). B^5B^5 birds were found to have both higher primary total and ME-resistant titers than B^2B^2 birds. Similar results were obtained with respect to total but not ME-resistant titers to SRBC. The authors proposed that these differences between MHC haplotypes may be a reflection of macrophage or T lymphocyte function. The effects seen in the BA system, also led the authors to suggest that the MHC genotype differences between the two lines may be associated with dissimilarities between the number of B cell clones capable of reacting with antigen.

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Eimeria tenella

General Features

E. *tenella* is an obligate intracellular parasite which causes cecal coccidiosis in chickens. It belongs to the phylum Apicomplexa as do the plasmodia. E. *tenella* has a complex life cycle, which contains both sexual and asexual stages (Figure 8). The exogenous stages of E. *tenella* are the oocysts, which are shed in the feces and undergo sporogony in the environment to generate sporozoites, enclosed within sporocysts. Following ingestion, oocysts undergo excystation, in the process releasing sporozoites into the intestinal lumen. Free sporozoites penetrate the intestinal epithelium of the ceca, which are two blind sacs appended to the junction of the colon and the ileum. Inside the cecal epithelium, sporozoites become rounded and transform into trophozoites. Trophozoites have the capacity to undergo merogony. Merogony is the production of merozoites through asexual proliferation. Merozoites are released into the gut lumen where they infect epithelial cells close to the point of release. After a few merozoite generations, gametogony takes place. Gametogony is the sexual proliferation stage of the life cycle. During this stage, merozoites enter cells and turn into either microgametocytes or macrogametocytes. Microgametocytes fertilize macrogametocytes to form a zygote, which surrounds itself with an oocyst wall. The oocyst is then released into the environment and the cycle occurs again (Jeurissen et al., 1996) [Fig. 8].

Cecal coccidiosis involves the destruction of the intestinal epithelium at the site of infection and eventually leads to death due to nutrient maladsorption and blood loss. The severity of the disease is estimated based on several parameters including weight loss,

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Figure 8. The life cycle of *Eimeria tenella*. The life cycle consists of both sexual and asexual stages. The parasite reproduces in the intestine of the chicken. Unsporulated oocysts are released into the environment where they sporulate and can infect new hosts. [Based on Jeurissen et al., 1996]
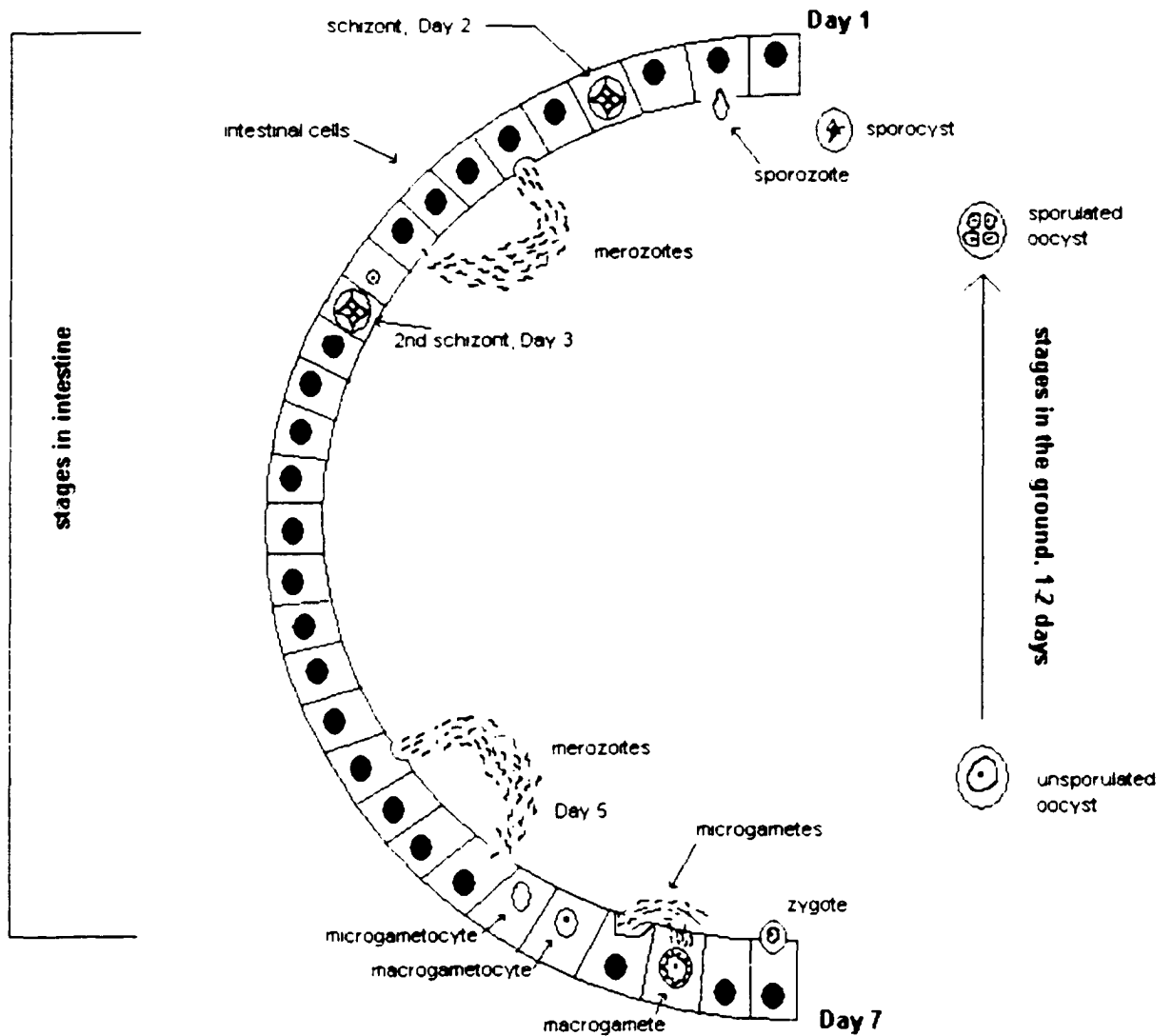
cecal lesions, fecal content, and mortality. Often these symptoms need to be considered simultaneously in order to evaluate the severity of the disease since they do not correlate well. Based on this observation, it has been suggested that there are at least three stages of immunity to cecal coccidiosis. One is characterized by complete resistance to the parasite, one is characterized by discharge of oocysts but no lesion occurrence, and one involves resistance to the clinical effects of the disease despite the presence of severe lesions (Long et al., 1980).

Immune Response

Initial studies on immunity to cecal coccidiosis date back to the 1940's when Rosenberg (1941) demonstrated genetic control of resistance to coccidiosis. In 1951, Edgar et al. showed that selection could lead to resistance to $E.$ $tenella$ infection. Later, resistant and susceptible lines were developed (Champion, 1954; Johnson and Edgar, 1982).

Lines selected for resistance and susceptibility to cecal coccidiosis have been used in order to characterize the genes affecting the outcome of the disease. Some of the earliest studies have focused on the B complex (Ruff and Bacon, 1989; Clare et al., 1985). Ruff and Bacon suggested that, while the B complex influences resistance and susceptibility to coccidiosis, it may play a minor role in the development of immunity to challenge infection, pointing to a difference in the identity of the genes controlling initial infections and those controlling subsequent development of immunity to further challenges. Ruff and Bacon found that, based on lesion score, the B^2B^2 genotype was associated with greater susceptibility to $Eimeria$ $acervulina$ but not $Eimeria$ $tenella$ infection than the B^5B^5 genotype. Clare et al. found that these differences were also

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evident with respect to immunity to cecal coccidiosis, the disease caused by *E. tenella*, using a repeated low dose immunization protocol, whereas Ruff and Bacon observed a greater immune protection in B^2 congenic lines than in B^5 congenic lines based on four 100-oocyst immunizations. The differences between the two studies with regard to the effect of the *B* system on immunity to the disease were explained by the fact that different immunization protocols were utilized. In support of this, Ruff and Bacon demonstrated that B^2B^2 congenic lines had low immunity to cecal coccidiosis following a single 100-oocyst immunization but a high degree of immunity following four 100-oocyst immunizations, while a B^5B^5 congenic line had a low level of protection using the same protocol. Furthermore, Clare et al. found evidence that the response to coccidia varied among genotypes depending on whether a nonviable oocyst antigen or a viable asexual stage antigen was used as a challenge agent. This suggested that the immune response to the disease may reflect not only the mechanism of antigen presentation but also the particular character of parasite antigens presented by the MHC.

In an attempt to identify the regions of the MHC contributing to differential resistance and immunity to *E. tenella*, Clare et al. (1989) studied resistance and susceptibility as well as immunity to cecal coccidiosis in *B-F/B-G* recombinant birds. The *B-F* region was implicated as a significant influence on disease susceptibility. Furthermore, the results of the study provided support to the proposition that different genetic mechanisms influence innate resistance to cecal coccidiosis on the one hand, and acquired immunity on the other. While $B\text{-}F^2$ birds showed greater resistance to the disease, after priming with the antigen they demonstrated little protection. Conversely, *B-*

F^{21} birds, while being susceptible to initial infection with the parasite, displayed enhanced protection following immunization.

The absence of a straightforward correlation between B haplotype effect on resistance to cecal coccidiosis on the one hand, and immunity to the disease on the other, pointed to genes other than the MHC as potential factors influencing the response to $E.$ *tenella* infection. Direct evidence for that was presented in 1989 when Lillehoj et al. found wide variations in innate resistance and acquired immunity to cecal coccidiosis between birds sharing a common genetic background but differing at their MHC as well as birds sharing a common B haplotype but differing with respect to their genetic backgrounds. Some of these nonMHC genes have been identified and include the A-E (Johnson and Edgar, 1984), I (Martin et al., 1986), and C (Johnson and Edgar, 1986) erythrocyte alloantigen systems.

Investigations regarding the cellular basis of immunity to $E.$ *tenella* infection are complicated by the fact that the parasite has different life cycle stages, each associated with the expression of various antigenic patterns. It is believed that the sporozoite is the invasive stage initiating infection in chicken cells and therefore it is the major immunogenic form of the parasite. The early studies focused on the potential of antibodies to mediate immunity to cecal coccidiosis. Antibodies which agglutinate, immobilize, or lyse various stages of the parasite have been identified in resistant chickens. Furthermore, sporozoites incubated with serum from resistant chickens were incapable of initiating an infection when injected into susceptible chickens (Huff and Clark, 1970). Of particular importance is IgA antibody, which constitutes the major portion of neutralizing antiserum found in the fecal contents of $E.$ *tenella* infected

chickens (Davis et al., 1977). Nevertheless, bursectomized birds still became immune to cecal coccidiosis, suggesting a contribution through mechanisms other than humoral immunity (Huff and Clark, 1970).

A direct link between cell-mediated immunity and the development of cecal coccidiosis was found through studies of the correlation between delayed hypersensitivity reactions and *in vitro* lymphocyte stimulation, as measurements of the strength of a cell-mediated immune response, and resistance to cecal coccidiosis. As a result of these studies, it was proposed that cell-mediated immunity is more important in disease resistance than amounts of circulating antibody, which fall below detectable levels after prolonged parasite persistence (Giambrone and Klesius, 1980). In a later study, however, it was shown that T lymphocyte deficient birds, as well as bursectomized birds, were capable of developing immunity to the disease, suggesting that the balance between the two may be conducive to optimal immunity but that either one can provide some protection without contribution from the other, possibly through alternative mechanisms (Rose and Hesketh, 1979). In support of this, it was shown that different immune mechanisms contribute to the development of immunity to *E. tenella* infection in normal versus bursectomized chickens. While the development of immunity in bursectomized birds involved a high proportion of T cell clones capable of activating cytotoxic activity in macrophages, immunity in normal chickens was associated with higher numbers of T cell clones interacting with primed B cells (Bhogal et al., 1986).

Studies correlating protective immune responses to coccidia and host genetic variation in T- and B-cell responses have pointed to a central role for cell-mediated immunity. T cells from resistant birds display higher lymphocyte proliferative responses

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following stimulation with sporozoite antigen *in vitro* than T cells from susceptible birds. In contrast, genetic variation with respect to antibody responses has not been noted. In fact, the relationship between resistance to infection and antibody responses appears inverse. The authors propose that in susceptible lines, the lack of protection may be a function of the tendency to initiate an immune response to irrelevant antigens or antigens mediating evasion of protective immunity (Bumstead et al., 1995).

In an attempt to evaluate the relative importance of T_h versus T_c lymphocytes in resistance and immunity to cecal coccidiosis, Breed et al. (1996) studied the changes in peripheral blood leukocyte T-cell subsets following infection with *E. tenella*. They observed a sharp transitory increase in the numbers of CD8⁺ T cell subsets and a gradual decrease of CD4⁺ T cells in the blood of infected birds following a primary challenge. Such effects were not seen following a secondary infection. These observations led the authors to propose that CD8⁺ cells may play a major role at the onset of immunity. The apparent decrease in the levels of CD4⁺ T cells could correlate with migration of these cells from the circulation to the ceca. The lack of observed changes in lymphocyte distribution following secondary infection is explained by an enhanced immune status already established in the cecum at the moment of secondary infection.

Studies of the differential role of T cell subsets in immunity to coccidial infection are complicated by the fact that CD8⁺ T cells, in addition to serving as mediators of immunity to the disease, also play an essential role as transporters of sporozoites to the crypt epithelium. Trout and Lillehoj (1995) found evidence for that phenomenon in the fact that CD8⁺-T- cell- depleted birds demonstrated a reduction in the number of oocysts following primary infection but an increase in the number of oocysts following secondary

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infection. The authors suggested that the decrease in the number of oocysts following primary infection is due to the lack of CD8⁺ T cells capable of transporting the parasite to the crypt epithelium, while the increase following secondary infection is illustrative of the role of CD8⁺ T cells in the development of immunity to reinfection. In contrast, CD4⁺ T-cell-depleted birds produced more oocysts than controls following primary but not secondary infection, suggesting that CD4⁺ T cells are important in the establishment of primary immunity. The differences between the findings of Breed et al. and Trout and Lillehoj could be a result of the dual function of CD8⁺ T cells as sporozoite transporters and mediators of immunity.

In conclusion, both CD4⁺ and CD8⁺ T cell subsets play a role in immunity to cecal coccidiosis. The balance between cell-mediated and humoral immunity is conducive to an optimal immune response since both branches of the immune system have been shown to contribute to immunity. The complex life-cycle of *E. tenella* as well as the capacity of the parasite to enter intestinal lymphocytes and be transported inside them to the site of infection complicate the study of the mechanisms involved in immunity.

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CHAPTER II

OBJECTIVES

The effect of the MHC on different immune parameters and disease susceptibility in chickens has been thoroughly studied. There exists a strong correlation between MHC haplotype and outcome of oncogenic virus infections, such as Rous sarcomas and Marek's disease, infections by eukaryotic parasites, such as cecal coccidiosis, bacterial infections, such as fowl cholera, autoimmune diseases, such as spontaneous autoimmune thyroiditis (SAT), and the outcome of *v-src*-induced tumors. Furthermore, MHC haplotype has been linked to immune parameters, such as antibody response, serum Ig levels, T lymphocyte function, and macrophage function.

The strong link between *B* complex haplotype and immune response in the chicken has been explained by the simplicity of the chicken MHC. The number of MHC genes within the complex is much lower than that in mammals. There is a tight linkage between these genes due to the compactness of the region, leading to transmission of haplotypes at loci encoded within the MHC as units. Furthermore, of the MHC I and MHC II genes, only one allele is expressed at high levels, while in mammals the expression of alleles is characterized by co-dominance. As a result, birds have a limited repertoire of specificities to deal with foreign antigens and rely on a "plus/minus" strategy to generate immune responses. This leads to a predominance in a population, of MHC haplotypes strongly selected by a particular virulent pathogen.

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The effect of the MHC on immune response is probably a reflection of differential antigen presentation associated with different haplotypes. However, the observation of phenotypic variation in lines identical at the MHC suggests a contribution from nonMHC loci to the development of immunity. Based on the polymorphism of the L system, as well as on previous studies suggesting a role for it in antibody response, fertility, bursa size, and outcome of Rous sarcomas, we hypothesized that $Ea-L$ represents one such locus.

We attempted to characterize the effect of $Ea-L$ on Rous sarcomas, antibody response to SRBC and BA, and cecal coccidiosis in fully segregating combinations of L alleles within different B complex backgrounds in order to investigate possible interactions between the two systems. Based on our findings we intended to propose a functional assignment for the L system. Our interests focused on potential confirmation and elucidation of the effect of L on Rous sarcomas and collection of evidence suggesting a mechanism through which it may exercise that effect, be it through modulation of the efficiency of antigen presentation, control of humoral responses, or definition of T lymphocyte function. Although these mechanisms are interdependent and their effects could not be separated clearly by the type of *in vivo* strategies we have adopted, still our results, together with determination of some molecular characteristics of the L antigen, could point to an analogous function in mammals or propose a novel immune function with implications for oncogenic or parasitic disease.

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CHAPTER III

ALLOANTIGEN SYSTEM *L* AFFECTS THE OUTCOME OF ROUS SARCOMAS

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ABSTRACT

This study was designed to examine the alloantigen system L effects on Rous sarcomas in three B complex genotypes. The parental stock were 50% Modified Wisconsin Line 3 x White Leghorn Line NIU 4 and 50% inbred Line 6.15-5. Pedigree matings of 2 B^2B^5 L^1L^2 sires to 5 B^2B^5 L^1L^2 dams per sire, produced experimental chicks segregating for B and L genotypes. Chicks were inoculated with 20 pock-forming units (pfu) Rous sarcoma virus (RSV) at six weeks-of-age. Tumors were scored six times over 10 weeks postinoculation after which the tumor scores were used to assign a tumor profile index (TPI) to each chicken. Tumor growth over time and TPI were evaluated by repeated-measures analysis of variance and analysis of variance, respectively. Six trials were conducted with a total of 151 chickens. The major histocompatibility (B) complex affected the responses as the B^2B^2 and B^2B^5 genotypes had significantly lower tumor growth over time and TPI than the B^5B^5 genotype. Separate analyses revealed no significant L system effect in B^2B^2 or B^2B^5 backgrounds. However, L genotype

significantly affected ($P < 0.05$) both tumor growth over time and TPI in B^sB^s chickens. B^sB^s L^1L^2 birds had TPI significantly lower than B^sB^s L^1L^1 chickens but not B^sB^s L^2L^2. Mortality was lower in the B^sB^s L^1L^2 birds than in B^sB^s L^2L^2 chickens. The L system, or one closely linked, affects the growth and ultimate outcome of Rous sarcomas. The response may depend upon the genetic background as well as MHC type.

INTRODUCTION

Genetic variation in antigenic determinants among members of a species produces alloantigens. Numerous alloantigens have been described based on immunogenetic analysis of antisera resulting from exchange of blood between individual animals. Chicken erythrocyte alloantigen systems A, B, C, D, E, H, I, J, K, L, N, P, and R (Dietert et al., 1992) have been identified by serological reactions or by other methods. The B blood group was one of the earliest alloantigen systems described (Briles et al., 1950). Schierman and Nordskog (1961) subsequently found that the B blood group was associated with skin homograft tolerance and thus established that the B system was the major histocompatibility complex (MHC) in the chicken. The B complex has a pivotal role in immune responsiveness and the outcome of pathogenic challenges (Dietert et al., 1991, Kaufman and Salomonsen, 1997).

Gilmour (1959) and Briles (1962) independently discovered the L alloantigen system and established that two haplotypes, L^1 and L^2, segregate. The L locus segregates independently from nine other erythrocyte alloantigen systems (Dietert et al., 1992) but has not been assigned to a linkage group. Alloantigen typing showed that the L system did not segregate (Crittenden et al., 1993) in a reference population established for

molecular mapping. In addition to the described L haplotype associations with responses to RSV-induced tumors, other investigations have revealed L allele frequency alterations following divergent selection for bursa size (Scott et al., 1988) and fertility changes among different L genotypes (DeSilva, 1965). Taylor and Briles (2000) examined the effect of eight nonMHC alloantigen systems on resistance and susceptibility to *Eimeria tenella* in both B^2B^2 and B^2B^5 backgrounds. The authors found an association only between the L system and cecal lesions. $B^2B^2L^1L^1$ chickens had higher lesion scores than $B^2B^2L^1L^2$ and $B^2B^2L^2L^2$ chickens. No significant L genotype effect was observed in a B^2B^5 background.

Rous sarcoma is a connective tissue tumor caused by the Rous sarcoma virus (RSV), an oncogenic RNA virus. Tumors develop after injection of the virus into susceptible chickens. The tumors may regress or progress depending on the level of antitumor immune response produced by the MHC (Collins et al., 1977; Schierman et al., 1977). Variation in RSV tumor outcome among identical MHC genotypes from crosses of inbred lines differing at the MHC (Collins et al., 1977, Brown et al., 1984, Plachy, et al., 1979, Plachy, J. 1984; Plachy, et al., 1984), different inbred lines identical at the B complex (Collins et al., 1980; Gilmour et al., 1983; Gilmour et al., 1986) or crosses of noninbred lines (Collins et al., 1979; Collins et al., 1985b) implicated a role for nonMHC genes. For example, nonMHC T lymphocyte alloantigens, Ly-4 and Th-1, and the B lymphocyte alloantigen Bu-1 interacted to alter the response against RSV tumors in crosses of B^2B^2 inbred lines (Gilmour et al., 1983; Gilmour et al., 1986).

Two previous studies found L alloantigen effects on the response to Rous sarcomas. Collins (1979) studied the effect of alloantigen systems $C, D, E, I,$ and L on

tumor outcome in the F_2 generation of inbred lines $6_3 \times 100$ that all had the B^2B^2 genotype. The C, D, E, and I systems did not influence tumor outcome. On the other hand, the L genotype significantly affected tumor growth in females but not in males. LePage et al. (2000b) examined the effect of alloantigen systems A, C, D, E, H, I, L, and P on Rous sarcoma outcome in two B complex genotypes: B^5B^5, a tumor progressor, and B^2B^5, a moderate progressor. Alloantigen systems A, C, D, E, H, I, and P had no significant effect on tumor fate. The L genotype correlated with a differential tumor outcome. In the B^2B^5 genotypic background, the L^1L^1 chickens had lower tumor size, tumor profile index (TPI), and mortality than the L^1L^2 chickens. Mortality was lower in L^1L^1 birds compared to L^1L^2 birds in the B^5B^5 background.

These earlier experiments indicate significant L alloantigen modulation of immune responses without complete B and L system segregation. The objective of this study is to further investigate L system effects on RSV-induced tumors. We used crosses producing progeny fully segregating for both the B complex and the L alloantigen. This structure allows examination of the L system effects in B genotypes that vary widely in their RSV-induced tumor outcome.

MATERIALS AND METHODS

Stock. Chickens for this study were derived from several lines. Line 6.15-5 is a congenic line (Dix and Taylor, 1996) that was produced by crossing USDA-ADOL inbred Line 15_1 (B^5B^5) to USDA-ADOL inbred Line 6_1 (B^2B^2). After ten backcross generations, heterozygous B^2B^5 chickens were mated *inter se* to produce Line 6.15-5 B^5B^5 birds that have 99.9% of the Line 6_1 genetic background. Modified Wisconsin line 3 is an experimental population derived from Wisconsin inbred Line 3 Ancona (95% inbred)

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[McGibbon, 1978], originally homozygous for genes of all alloantigen loci except the B system. The modification consisted of introducing alloantigens for selected systems from White Leghorns and backcrossing two or more generations to Line 3. White Leghorn Line NIU 4 was derived over 20 generations from inter se matings of crosses between four commercial parent stocks and selecting for equal frequencies of alloantigens segregating at 9 alloantigen loci.

Line 6.15-5 (B^5B^5 L^1L^1) dams were mated to B^2B^2 L^1L^2 sires from a line cross between modified Wisconsin Line 3 Ancona x White Leghorn line NIU 4 sires (B^2B^2 L^1L^2) as described by LePage et al. (2000b). Chickens from this mating that had the B^2B^5 L^1L^2 genotype contained 50% of the Line 6.15-5 genome and were used as parents to produce the experimental progeny. Pedigree matings of 2 B^2B^5 L^1L^2 sires to 5 B^2B^5 L^1L^2 dams per sire, produced 6 hatches having one hundred and fifty one chicks segregating for all possible combinations of B and L genotypes. The birds were hatched at the University of New Hampshire Poultry Research Farm and were wing-banded for identification. Vaccinations against Marek's disease and Newcastle-bronchitis were administered at hatch and 10 d, respectively. The chicks were housed in heated brooder batteries with water and food freely available. Six-week-old chicks were transferred to isolation cages for the remainder of the experiment.

Alloantigen typing. The chickens were typed for B and L systems in agglutination assays utilizing antisera specific for the haplotypes of the parental stocks (Briles and Briles, 1982). When chicks reached 3 wk of age, 0.5 mL blood was drawn from the wing vein and added to cold sodium citrate anticoagulant solution (68 μM sodium citrate / 72 μM sodium chloride). Samples were shipped overnight with ice packs

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to Northern Illinois University. Fifty μL of a 2% suspension of washed red blood cells was dispensed into tubes containing 100μL of antiserum specific for the *B* and *L* system haplotypes of interest. Following a 2 hr room temperature incubation, the reaction mixtures were transferred to 3° C for an overnight incubation. The following day, cells were resuspended and scored visually for agglutination after a 1 hr incubation at room temperature.

RSV Challenge and Tumor Evaluation. At six weeks of age, the birds were inoculated in the right wingweb with 20 pfu of the Bryan high titer strain of Rous sarcoma virus (RSV). [RAV-1]. subgroup A. Two weeks following RSV challenge, tumors were scored for size using the following scale: 0 = no palpable tumor; 1 = small tumor up to 0.5 cm diameter; 2 = tumor > 0.5 up to 1.2 cm diameter; 3 = tumor > 1.2 up to 1.2 wingweb area; 4 = tumor > 1/2 wingweb area, but < entire wingweb area; 5 = tumor filling the entire wingweb; 6 = massive tumor extended beyond wingweb; and 7 = death during the experiment (Collins *et al.*, 1977). Tumor size was also scored at weeks three, four, six, eight, and ten post-inoculation for a total of six tumor size scores over the ten week experimental period. The six tumor size scores were then used to assign a tumor profile index (TPI) to each bird as an indicator of the tumor growth pattern. The TPI values were those of Collins et al., (Collins et al., 1985b), where 1 = complete regression by 70 d post-inoculation, or a decreasing slope, or complete regression by 56 d followed by recurrence; 2 = general upward trend, or plateau; slight regression after 56 d; 3 = terminal tumor after 42 days post-inoculation; 4 = terminal tumor between 29 and 42 d post-inoculation; and 5 = terminal tumor by 28 d post-inoculation.

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Statistical Analysis. Tumor scores were analyzed by repeated measures ANOVA with hatch, sex, sire, dams within sire, time, B genotype, L genotype, and a B x L interaction as main effects. A large effect of B type led to separate analyses for each B genotype having hatch, sire, dams within sire, time, and L type as main effects. The TPI values were rank transformed and analyzed by ANOVA as described by Conover and Iman (Conover and Iman, 1981) with the same independent variables except time as in the repeated measures ANOVA. Significant differences between alloantigen system genotypes were determined using Fisher's protected LSD. Mortality rates were evaluated using chi-square analysis.

RESULTS

Alloantigen system genotypes for B and L segregated independently of each other in the 151 progeny. Repeated measures analysis of tumor score revealed a significant ($P = 0.0001$) B genotype effect on tumor growth. The significant ($P = 0.0001$) interaction between time and B genotype indicated that tumor growth differed over time as a function of B genotype (Fig. 9A). Analysis of the TPI values demonstrated a highly significant B genotype effect ($P = 0.0001$). The highest mean TPI, found in the B^5B^5 genotype (n = 31; 3.35 ± 0.18) was significantly greater than the TPI of B^2B^2 birds (n = 46; TPI = 1.43 ± 0.16) and B^2B^5 birds (n = 74; TPI = 1.36 ± 0.10) [Fig. 9B]. The B genotype significantly affected ($P = 0.0001$) mortality rates of 13.1%, 10.8%, and 77.4% for the B^2B^2, B^2B^5, and B^5B^5 genotypes, respectively (Table II, Analysis 1). No differences between males and females were detected.

The overwhelming B genotype effect on tumor outcome led to separate analyses of L genotype effects on tumor score, TPI, and mortality within each specific B genotype.

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Therefore, L^1L^1, L^1L^2, and L^2L^2 birds were analyzed within the B^2B^2, B^2B^5, and B^5B^5 MHC types. No significant effect of L genotype on tumor score over time, TPI, or mortality was evident in either the B^2B^2 or B^2B^5 (data not shown) MHC genotype backgrounds. Both the B^2B^2 and B^2B^5 genotypes from this mating exhibited strong regression of RSV-induced tumors.

The L genotype, however, exerted a significant influence on tumor score, TPI, and mortality in the B^5B^5 genotypic background. Repeated measures analysis of tumor score indicated a significant change in tumor size over time ($P = 0.0001$) as well as a significant L genotype x time interaction ($P = 0.00017$). The overall pattern of tumor growth was an increase in tumor size 2, 3, and 4 weeks post-inoculation followed by a lower rate of tumor size increase in B^5B^5 L^1L^2 (n = 14) compared with either the B^5B^5 L^1L^1 (n = 5) or B^5B^5 L^2L^2 (n = 12) genotypes (Fig. 10A). Tumor size at 10 weeks post-inoculation was diminished in the L^1L^2 genotype (5.43 ± 0.6) compared to either the L^1L^1 (7.00 ± 0.0) or L^2L^2 (6.83 ± 0.2) genotypes.

Analysis of variance of the TPI values also found a significant L genotype effect on tumor outcome ($P = 0.023$) in B^5B^5 chickens. The L^1L^2 genotype had TPI of 2.93 ± 0.3 which was significantly lower than the TPI values of L^1L^1 (4.00 ± 0.3) but not L^2L^2 (3.58 ± 0.3) chickens (Fig. 10B). Furthermore, chi-square analysis of mortality differences revealed a significant L effect ($P = 0.046$). The L^1L^2 genotype had the lowest mortality rate (57.1%), whereas the L^2L^2 and L^1L^1 genotypes had mortality rates of 91.7% and 100% respectively (Table II, Analysis 2).

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DISCUSSION

Segregating combinations of two B and two L haplotypes produced nine different genotype combinations in the experimental progeny. The results are consistent with the differential B^2B^2 and B^5B^5 genotype effects on retroviral oncogene tumors reported previously. B^2B^2 chickens regress RSV tumors or v-src DNA tumors whereas B^5B^5 hosts progress these tumors (Collins et al., 1977, Taylor et al. 1992). The same divergent B complex responses are evident in tumor metastasis (Collins et al., 1986, Taylor et al., 1994) as well as in immunity to a second inoculation of RSV or v-src DNA (Taylor et al., 1994; Guyre et al., 1982). Other B complex haplotypes also differ in their responses to RSV tumors or v-src DNA tumors (Plachy et al., 1986, Svoboda et al., 1992; Plachy et al., 1994). Heterozygous B^2B^5 chickens usually have less tumor regression than B^2B^2 chickens (Collins et al., 1977; Collins et al., 1986). The current study assessed different mating types compared with former experiments so the profound similarity in B^2B^2 and B^2B^5 genotypes' tumor growth may be due to nonMHC genes.

The L alloantigen genotypes were analyzed within each B genotype to eliminate the possibility that the powerful B complex effect would overcome any L system effects. No significant L effect was evident in either the B^2B^2 or B^2B^5 genotypic background as both genotypes regressed tumors. A significant L influence, however, was evident in the B^5B^5 background. This result suggests that the robust tumor regression found in B^2B^2 and B^2B^5 birds masks the weak L effect, which becomes obvious only in the B^5B^5 progressor background. Based upon tumor growth over time, TPI, and mortality, the L^1L^2 genotype mitigated the progressive effect of the B^5B^5 genotype and did so significantly compared to the L^1L^1 genotype.

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Two prior studies examined the L alloantigen system influence on Rous sarcomas. Among the F_2 generation of B^2B^2 inbred lines 6_3 x 100, tumor fate was affected significantly by L genotype (Collins 1979). Females of the L^1L^1 genotype had significantly lower TPI than the L^2L^2 genotype. No L genotype effect was found in males. LePage et al. (2000b) tested B^2B^5 and B^5B^5 progeny that segregated for at least two alleles of eight non-B alloantigen systems. Significant L alloantigen effects on Rous sarcomas were found in both B complex backgrounds. The L^1L^1 genotype was associated with lower tumor scores, TPI, and mortality than the L^1L^2 genotype in a B^2B^5 background. Lower mortality was found in B^5B^5 L^1L^1 chickens than in B^5B^5 L^1L^2 chickens.

The apparent disagreement of the present data with previous studies may be attributed to several factors. First, each study used a mating type with a different genetic background. Collins (1979) used progeny from the cross of two inbred lines, LePage et al. (2000b) used progeny that were 50% Modified Wisconsin Line 3 and 50% White Leghorns, whereas the current study used progeny that consisted of 50% inbred line 6-15.5 and 50% Modified Wisconsin Line 3 x NIU 4 White Leghorns. Second, LePage et al. (2000b) used a higher virus dose (30 pfu) than this experiment (20 pfu). Virus dose may influence tumor growth in progressive genotypes (Vincent and Taylor, 1988), such as B^5B^5. Third, the current study is the only one that utilizes full segregation of B and L haplotypes. Effects of background genes, other than B and L, cannot be completely excluded by the results.

Growth and subsequent division of tumor cells as well as cellular recruitment via viral-replication genes (Taylor et al., 1992) affect RSV tumor growth. T cells are

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principally responsible for RSV tumor regression (Cotter et al., 1976, Gilmour et al., 1983, Plachy et al. 1984, Powell et al., 1987). Cross-reactions between tumor antigens and certain MHC haplotypes may impinge on antigen recognition. The B_5 antigen cross-reacted to one or more RSV tumor antigens because Rous sarcoma progression increased in B^2B^2 chickens previously made tolerant to the B_5 antigen from progressor chickens compared with untreated B^2B^2 controls (Heinzelmann et al., 1981a; Heinzelmann et al., 1981b). Tumor regressor Line CB $B^{12}B^{12}$ chickens progressed a transplantable v-src-induced tumor after they were made tolerant to Line CC B^4B^4 or CB.R1, (B-F^{12} B-G^4). This result supported a cross-reaction between the B_4 antigen and a v-src tumor antigen (Plachy et al., 1994).

The L^1L^2 genotype advantage in B^5B^5 tumor outcome indicates complementation. The host response to a tumor is a complex reaction to a multitude of antigens (Brown et al., 1982). Particular heterozygous combinations of MHC molecules may facilitate tumor or viral antigen recognition (Lukacs et al., 1989). Compared with the homozygotes, a heterozygote may complement through either improved recognition efficiency of the same number of antigens or increased recognition of additional antigens. The L^1L^2 genotype or closely linked genes, in B^5B^5 chickens of the current study, may have enhanced the immune response against RSV tumors by interacting with an effector molecule, facilitating viral or tumor antigen interaction with effector molecules or partially overcoming the B_5 antigen-associated tolerance to tumor antigens. These effects might be accomplished through increased T cell activation, B complex antigen expression, or both.

Plachy (1985) described MHC and nonMHC gene complementation in crosses of the Prague congenic inbred lines, CB ($B^{12}B^{12}$), CB.I (B^7B^7), and inbred line IA (B^7B^7). Chickens of the $B^{12}B^7$ genotype from either CB x CB.I or CB x IA matings had more regressing tumors than either $B^{12}B^{12}$ or B^7B^7 chickens indicating complementation between the B^{12} and B^7 haplotypes. In addition, greater tumor regression was evident in the $B^{12}B^7$ genotype CB x IA chickens than in the same genotype from the CB x CB.I mating denoting a nonMHC gene effect that was complementary. Matings of Line CC (B^4B^4) with CB.I and IA revealed similar MHC and nonMHC complementation that was independent of age. B^4B^7 chickens from the CC x IA mating had more tumor regression than CC x CB.I cross (Plachy 1985).

Other nonMHC genes have demonstrated complementary effects on the outcome of Rous sarcomas. Gilmour et al. (1983) studied progeny derived from inbred line crosses identical for the MHC (B^2B^2) but segregating for Ly-4 and Th-1 nonMHC T-cell surface antigen genes. A homozygous/heterozygous interaction was found in that Ly-4^aLy-$4^a/Th$-1^aTh-1^b and Ly-4^aLy-$4^b/Th$-1^aTh-1^a genotypes had increased RSV tumor regression. The interaction between the a and b haplotypes occurred when the other locus was the aa genotype. Another example of complementation occurred between nonMHC B and T cell alloantigens, Bu-1 and Ly-4. Progeny from a different cross of B^2B^2 inbred lines had greater RSV tumor regression due to complementation between the Ly-4^a and Bu-1^b alleles or the Ly-4^b and Bu-1^a alleles (Gilmour et al., 1986).

Genes other than the MHC can affect the RSV tumor progressive B^5B^5 genotype. Collins et al. (1985a) examined Rous sarcoma metastasis in B^5B^5 hosts from two populations: (Line 6_1 x Line 15_1)F_5 White Leghorn cross and (Line 6_1 x Line 15_1)F_5

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Leghorn x Line UNH 105 New Hampshire F_2. The incidence of tumor metastasis was significantly lower in the $B^s B^s$ White Leghorn x UNH 105 cross than in the $B^s B^s$ White Leghorn population, suggesting a possible nonMHC background effect on metastasis. Another study found that $B^s B^s$ birds having alloantigen haplotypes D^{1} or F^{R} had significantly lower TPI than those with D^{1} or F^{R} (LePage et al. 2000b). Mortality in $B^s B^s$ chickens was lowered by the $L^1 L^1$ genotype compared with the $L^1 L^2$ genotype (LePage et al. 2000b). However, that study used chickens with a different genetic background and used two B genotypes with three L genotypes compared to the current research that has full segregation of B and L genotypes.

The present experiments revealed that the B complex and the L alloantigen system significantly affected tumor growth over time, TPI, and mortality due to RSV-induced tumors. This work also adds unique information regarding L alloantigen effects on the immune response to Rous sarcomas in the context of B^2 and B^s haplotype segregation. Complementation by the $L^1 L^2$ genotype in the $B^s B^s$ birds supports the conclusion that the L alloantigen system, or some closely linked gene(s), has significant effects on the fate of Rous sarcomas. Further research should examine possible interactions between the L system, different B haplotypes and other nonMHC genes.

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Figure 9. Effect of B genotype on (A) tumor size scores over a 10-wk experimental period and (B) tumor profile indices (TPI) in B^2B^2 (n = 46), B^2B^5 (n = 74) and B^5B^5 (n = 31) chicks inoculated with 20 pfu Rous sarcoma virus. Tumor growth as a function of B genotype differs significantly (P = 0.0001) over time. Bars having no common letter differ significantly (P < 0.05).

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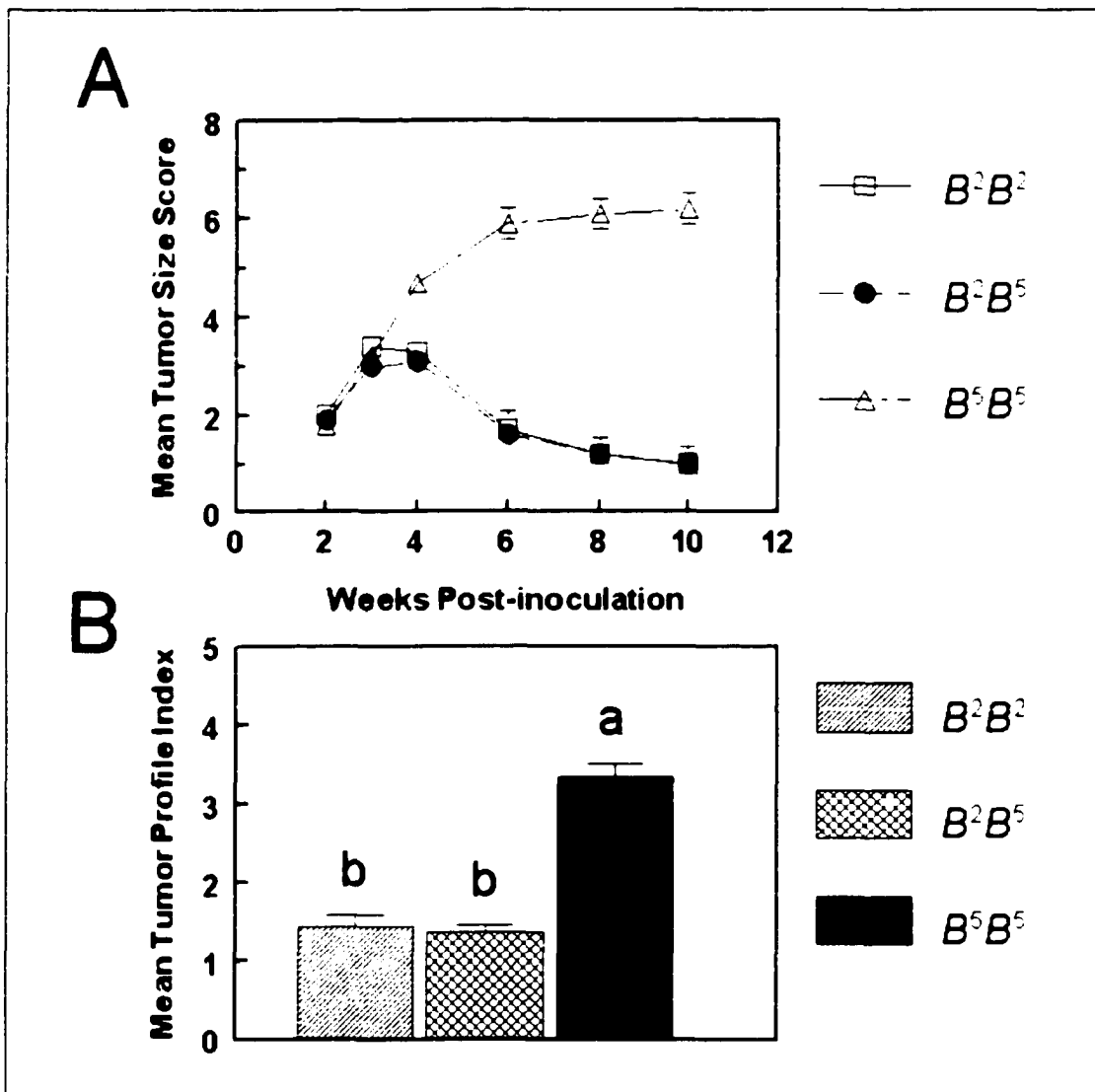
Figure 10. Effect of L alloantigen genotype on (A) tumor size scores over a 10-wk experimental period and (B) tumor profile indices (TPI) in B^5B^5 L^1L^1 (n = 5), B^5B^5 L^1L^2 (n = 14) and B^5B^5 L^2L^2 (n = 12) chicks inoculated with 20 pfu Rous sarcoma virus. Tumor growth as a function of L genotype differs significantly ($P = 0.00017$) over time. Bars having no common letter differ significantly ($P < 0.05$).

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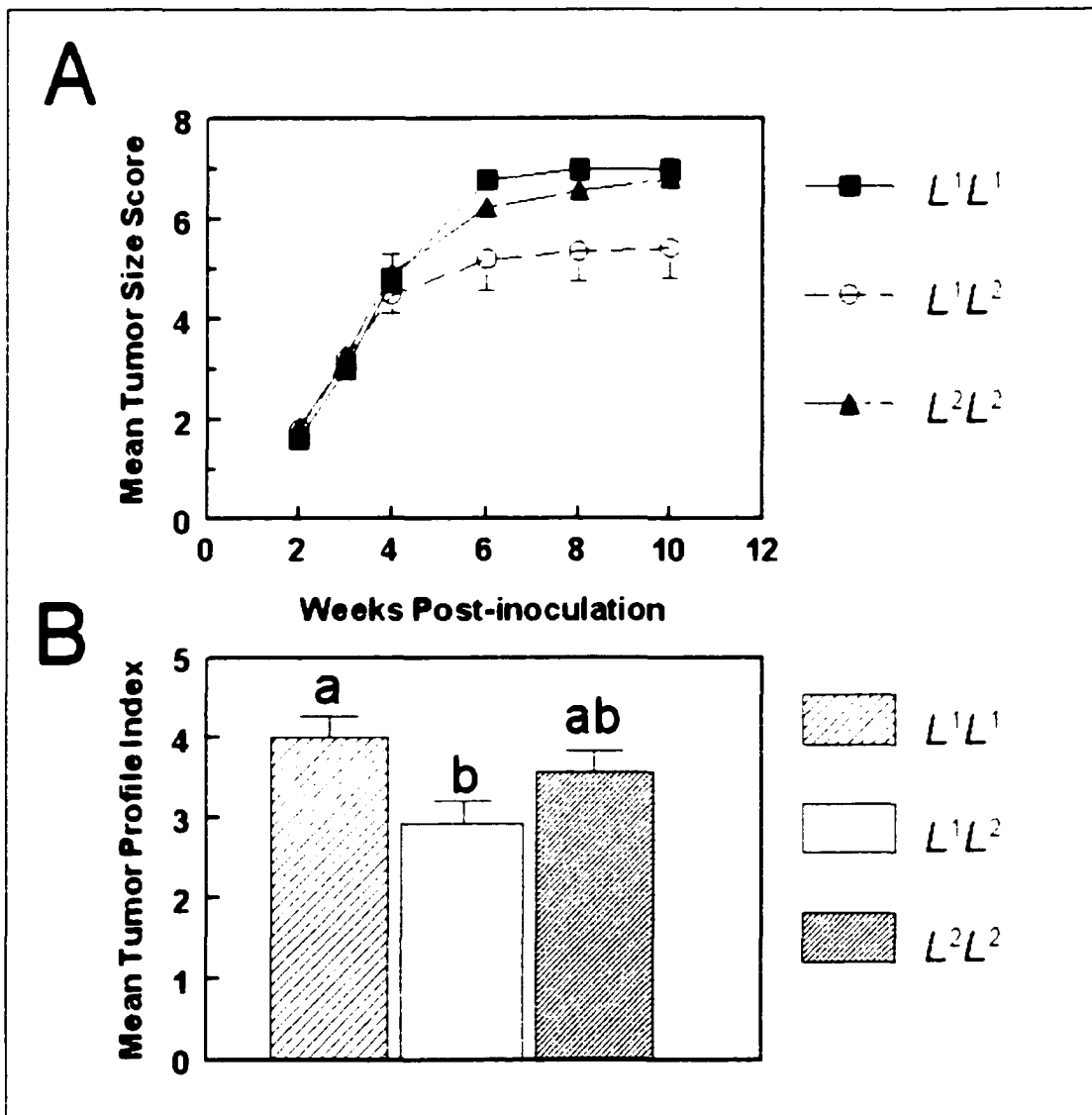
Table II. Contingency table chi-square analyses of mortality due to Rous sarcomas among different genotypes in modified Wisconsin Line 3 x 6.15-5 White Leghorn chickens.

Analysis	Genotype	Alive		Dead		X^2	P
		N	%	n	%		
1	B^2B^2	40	86.9	6	13.1	56.63	0.0001
	B^2B^5	66	89.2	8	10.8		
	B^5B^5	7	22.6	24	77.4		
2	$B^5B^5 L^1L^1$	0	0	5	100	6.14	0.046
	$B^5B^5 L^1L^2$	6	42.9	8	57.1		
	$B^5B^5 L^2L^2$	1	8.3	11	91.7		

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CHAPTER IV

ALLOANTIGEN SYSTEM L AFFECTS ANTIBODY RESPONSES

Zdravka Medarova, W. Elwood Briles, and Robert L. Taylor, Jr.

ABSTRACT

Alloantigen system L is a polymorphic protein expressed on the surface of chicken erythrocytes and possibly certain leukocyte subpopulations. Previous studies demonstrated that the L system affects Rous sarcoma outcome and phagocytic function. The present experiments examined the L system influence on antibody responses to two antigens: SRBC (T-dependent) and *Brucella abortus* (BA, partially T-independent) in three B complex genotypes. The parental stock were 50% Modified Wisconsin Line 3 x White Leghorn Line NIU 4 and 50% Inbred Line 6-15.5. Pedigree matings of 4 $B^2B^5L^1L^2$ sires to 5 $B^2B^5L^1L^2$ dams per sire produced three hatches (n = 183) for the antibody response to SRBC and two hatches (n = 198) to study antibody response to BA. At 4 and 11 weeks of age the experimental birds were injected intravenously with 1 mL of 2.5% SRBC or 0.1 mL of 10% BA. Total and mercaptoethanol (ME)-resistant primary and secondary titers were analyzed by least squares ANOVA. Alloantigen L had a significant effect on total primary antibody titer to SRBC in a B^5B^5 background (p < 0.004) and on

total (p < 0.011) and ME-resistant (p < 0.017) secondary titer to SRBC in the B^2B^5 genotype. Total (p < 0.004) and ME-resistant (p < 0.005) secondary titers to BA in B^2B^5 chickens were significantly affected by alloantigen L. The data indicate that the alloantigen L locus or genes in the immediate chromosomal vicinity affect antibody responses to SRBC and BA.

INTRODUCTION

Alloantigen L is one of thirteen described systems expressed on the surface of chicken erythrocytes. Chicken leukocytes also appear to express this alloantigen on their surface (W. E. Briles, Department of Biological Sciences, Northern Illinois University, DeKalb, IL 60115, personal communication) but these data were not conclusive. The L system is polymorphic with L^1 and L^2 alleles (Gilmour 1959, Briles, 1962). Both the L^1 and L^2 alleles have been determined to encode antigens of approximately 135kD (Ameiss and Briles, 2000). Previous research has shown that allele combinations of the L system influence a range of immune responses including the fate of Rous sarcoma virus (RSV)-induced tumors (LePage et al., 2000b), phagocytosis (Qureshi et al., 2000) and response to *Eimeria tenella* (Taylor and Briles, 2000).

LePage et al. (2000b) studied segregating combinations of genes encoding eight alloantigen systems for their effects on Rous sarcomas in both B^2B^5 and B^5B^5 MHC backgrounds. Among these alloantigens, L alleles were the only system that affected Rous sarcoma growth. The L^1L^1 genotype was associated with a stronger anti-sarcoma response, manifested as lower tumor score, TPI, and mortality in the B^2B^5 background compared with the L^1L^2 genotype. In the B^5B^5 background, the L^1L^1 genotype had lower mortality than the L^1L^2 genotype. Medarova et al. (2002) examined the effect of fully

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segregating combinations of L system alleles on Rous sarcomas in three B complex genotypes: B^2B^2, B^2B^5, and B^5B^5. That study found an L effect only in a B^5B^5 genetic background. The L^1L^2 genotype was associated with a lower tumor score, TPI, and mortality than the L^1L^1 and L^2L^2 genotypes. The strong anti-sarcoma response in the B^2B^2 and B^2B^5 backgrounds masked L effects in those genotypes. The differences between these two studies in the observed L alloantigen effects were attributed to the different genetic backgrounds of the experimental chickens and different RSV doses.

Qureshi et al. (2000) analyzed the effect of alloantigen systems on phagocytosis following *in vitro* incubation of monocyte monolayers with viable *E. coli*. The L system had a significant effect on monocyte function that was independent of the B system. In 4 wk old birds, the L^1L^1 genotype had a significantly higher percentage of phagocytic monocytes than the L^1L^2 genotype. Other experiments found no L alloantigen effect on macrophage nitrite or IL-6 production (M. A. Qureshi, Department of Poultry Science, N. C. State University, Raleigh, NC 27695, personal communication).

Taylor and Briles (2000) examined alloantigen systems for their effects on resistance and susceptibility to *E. tenella* in B^2B^2 and B^2B^5 backgrounds. Only the L system affected the response among the eight alloantigen systems studied. The effect on cecal lesion scores was evident in a B^2B^2 background but not in a B^2B^5 background because $B^2B^2L^1L^1$ chickens had higher lesion scores than $B^2B^2L^1L^2$ and $B^2B^2L^2L^2$ chickens.

Selection for bursa size or antibody response to SRBC altered L allele frequencies. Scott et al. (1988) found changes in L alloantigen allele frequencies in chickens divergently selected for bursa size for 21 generations. The L^2 allele had an

average frequency of 0.02 in the Large Bursa Line (LBL), whereas the Small Bursa Line (SBL) had an average frequency of 0.54. The base line was fixed for L^1, which occurred after selection was initiated. The SBL had significantly higher primary and secondary antibody against SRBC compared with the LBL. In contrast, serum IgG was lower in the SBL than in the LBL (Landreth and Glick, 1973; Yamamoto and Glick, 1982). No causal relationship between L allele frequency changes and these immune effects was demonstrated, but the results were not entirely due to the B complex, suggesting nonMHC gene effects (Landreth and Glick, 1973; Yamamoto and Glick, 1982). Rees and Nordskog (1980) found significant differences in basal serum IgG levels among inbred lines but no differences between IgG levels according to B haplotypes segregating within these lines, providing further support for nonMHC gene effects on IgG.

L allele frequencies were shifted through selection for primary antibody response to SRBC (Dunnington et al., 1984; Martin et al., 1990). In the High Antibody Line and the Low Antibody Line, the L^2 allele was fixed despite segregation for both L^1 and L^2 alleles in the Cornell randombred stock, which formed the base population. A founder effect could not be excluded as contributing to the L frequency changes. In both studies, selection for high or low antibody response shifted B haplotype frequencies indicating an MHC role in antibody response to SRBC.

Based on these studies, we hypothesized that the L system modulates, directly or indirectly, the potential for producing an effective antibody response to foreign antigens. We examined the L alloantigen effect on primary and secondary responses to SRBC and BA in the identical line of chickens used by our laboratory to study the L system's role in Rous sarcoma outcome (Medarova et al., 2002). These chickens have two L alloantigen

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alleles fully segregating with two B complex haplotypes to minimize the influence of other background genes and to reveal interrelationships between L and B effects.

MATERIALS AND METHODS

Stock. Chickens for this study were derived from several inbred lines. Congenic Line 6.15-5 (Dix and Taylor, 1996) was produced by crossing USDA-ADOL inbred Line 15_1 (B^5B^5) and Line 6_1 (B^2B^2) followed by ten backcross generations to Line 6_1. Heterozygous B^2B^5 chickens from the tenth backcross generation were mated *inter se* to produce Line 6.15-5 B^5B^5 birds that have 99.9% of the Line 6_1 genetic background. Modified Wisconsin Line 3 is an experimental population derived from Wisconsin inbred Line 3 Ancona (95% inbred) [McGibbon, 1978], produced by introducing selected alloantigen system alleles from White Leghorns and backcrossing two or more generations to Line 3. White Leghorn Line NIU 4 was derived over 20 generations from *inter se* matings of crosses between four commercial parent stocks and selecting for equal frequencies of alloantigens segregating at 9 alloantigen loci.

Modified Wisconsin Line 3 x Line NIU 4 sires ($B^2B^2L^1L^2$) were crossed to inbred Line 6.15-5 dams ($B^5B^5L^1L^1$) to produce the parental stock consisting of 50% inbred Line 6.15-5 with the $B^2B^5L^1L^2$ genotype. Pedigree matings of 4 sires to 5 dams per sire produced experimental progeny segregating for all possible combinations of B and L haplotypes. Three hatches having 183 chickens were used for SRBC injection and two hatches having 198 progeny were injected with BA. The birds were hatched at the University of New Hampshire Poultry Research Farm and were wing-banded for identification. Vaccinations against Marek's disease and Newcastle-bronchitis were administered at hatch and 10 d, respectively. The birds were housed in heated brooder

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batteries with water and food freely available. Six-week-old chicks were transferred to isolation cages for the remainder of the experiment.

Alloantigen typing. The chickens were typed for *B* and *L* systems in agglutination assays utilizing antisera specific for the haplotypes of the parental stocks (Briles and Briles, 1982) as described (LePage et al., 2000b). After the chicks reached 3 wk of age, 0.5 ml blood was drawn from the wing vein to cold sodium citrate anticoagulant solution (68 μM sodium citrate / 72 μM sodium chloride). Samples were shipped overnight with ice packs to Northern Illinois University. Fifty μl of a 2% suspension of washed red blood cells was dispensed into tubes containing 100μl of antiserum specific for the *B* and *L* system haplotypes of interest. Following a 2-hr room temperature incubation, the reaction mixtures were transferred to 3°C for an overnight incubation. The following day, cells were resuspended, incubated for 1 hr at room temperature, and scored visually for agglutination.

Antigen Inoculation. At 4 weeks of age, the experimental birds were injected intravenously with 1 mL of a 2.5% solution of SRBC[3] in Alsever's solution. A different group of segregating chicks was injected with 0.1 mL of a 10% solution of *Brucella abortus* standard tube test antigen[4]. Blood samples were taken 7 d post-injection. Serum from SRBC and BA inoculated birds was separated and stored for the titer assay. Each of the procedures was replicated in the same birds at 11 weeks of age to assay for secondary total and ME-resistant antibody titers to the specific antigens.

Antibody Titration. Standard microtiter techniques were used to assay for levels

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[3] Charles River Pharmservices, Southbridge, MA 01550

[4] National Veterinary Services Laboratories, Ames, IA 50010

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of serum antibodies to SRBC. Total anti-SRBC antibody levels were assayed according to the method described by Wegman and Smithies (1966). Total BA antibody was assayed according to the procedure described by McCorkle and Glick (1980). Mercaptoethanol (ME)-resistant antibody (IgG) levels to SRBC and BA were determined according to the method described by Yamamoto and Glick (1982). Titer was expressed as the $\log_2$ of the reciprocal of the highest dilution associated with a visible agglutination.

Statistical Analysis. The total and ME-resistant antibody titers for the primary and secondary response were analyzed by least squares ANOVA with hatch, sire, dams within sires, B genotype, and L genotype as main effects. A B x L interaction was included. The data were subdivided by B genotype and analyzed with hatch, sire, dam(sire), and L genotype as main effects. Significant means were separated by Fisher's protected LSD.

RESULTS

There was no significant B genotype effect on either the primary or secondary total or ME-resistant antibody response to SRBC. The data were divided by B genotype and analyzed for an L alloantigen effect. A significant L effect (p < 0.004) on the total primary antibody response to SRBC within a $B^{s}B^{s}$ background (n = 38) was found. In these birds, the $L^{1}L^{2}$ genotype was associated with the lowest total primary titer (5.38 ± 0.34). That titer was significantly lower than the titers of the $L^{2}L^{2}$ (6.67 ± 0.56) and $L^{1}L^{1}$ (6.6 ± 0.47) birds (Fig. 11).

Analysis also revealed a significant L influence on both total (p < 0.011) and ME-resistant (p < 0.017) secondary antibody response to SRBC within a $B^{2}B^{s}$ genotypic background. Among the $B^{2}B^{s}$ genotype (n = 99), the total secondary antibody titer to

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SRBC in the L^1L^1 birds was significantly lower (7.6 ± 0.35) than the L^1L^2 (7.98 ± 0.31) and L^2L^2 (7.94 ± 0.27) birds (Fig. 12). The B^2B^5 L^1L^1 birds also had a significantly lower ME-resistant secondary antibody titer to SRBC (6.5 ± 0.37) than either the B^2B^5 L^1L^2 (7.0 ± 0.25) or B^2B^5 L^2L^2 (7.0 ± 0.21) genotypes (Fig. 12).

The analysis of antibody response to *Brucella abortus* revealed no significant *B* system effect on either primary or secondary, total or ME-resistant titer. The data for each *B* genotype were analyzed for *L* system effects. The *L* genotype significantly affected total (p < 0.004) and ME-resistant (p < 0.005) secondary titer to BA within a B^2B^5 genotypic background (n = 100). The L^1L^1 genotype had the highest total secondary titer to BA (7.58 ± 0.12) that differed significantly from L^2L^2 birds (6.6 ± 0.07) but not L^1L^2 (7.13 ± 0.05) birds (Fig 13). The ME-resistant secondary titer to BA was also significantly higher in the B^2B^5 L^1L^1 genotype (6.96 ± 0.12) than in the B^2B^5 L^2L^2 (6.1 ± 0.08) but not the B^2B^5 L^1L^2 (6.77 ± 0.06) genotype (Fig 13).

DISCUSSION

The segregating combinations of the B^2 and B^5 haplotypes did not affect the antibody response to either antigen. Dix and Taylor (1996) found *B* complex effects on total and ME-resistant primary antibody against SRBC and BA in which congenic Line 6-15.5 (B^5B^5) had significantly higher antibody than congenic Line 6-6.2 (B^2B^2). On the other hand, Bacon et al., (1987) showed no difference in anti-SRBC or BA antibody titers between congenic lines 15.6-2 (B^2B^2) and 15.15-5 (B^5B^5) having the Line 15I$_5$ genetic background. Three points address these divergent results. First, the two congenic series have different genetic backgrounds (15I$_5$ vs. 6$_1$). Second, the 15.*B* lines had undergone

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five backcross generations at the time of testing, suggesting that other genes may have affected the responses. Finally, Bacon et al., (1987) injected the SRBC and BA antigens simultaneously, which may have modulated the response. The lack of a B complex effect in the current study suggests that background genes may have affected the antibody response.

The only significant L effect on primary antibody response was against SRBC within a B^5B^5 background. Since there was no significant B complex effect, the L effect did not occur in the B genotype (B^5B^5) that would have been expected to be the highest based on the results of Dix and Taylor (1996). The L^1L^2 genotype had significantly lower total primary antibody compared with either of the homozygous L genotypes. Qureshi and Taylor (1993) discovered that macrophages from congenic Line 6-15.5 (B^5B^5) had significantly higher SRBC phagocytosis than congenic Line 6-6.2 (B^2B^2). Qureshi et al. (2000) found that the L^1L^1 genotype had significantly greater monocyte phagocytosis of $E. coli$ than the L^1L^2 genotype, an effect that was independent of the B system. The L^2L^2 genotype was not produced in that study. The absence of a B effect on the anti-SRBC response argues against differential phagocytosis by different B genotypes in the current study. Lower SRBC phagocytosis by the L^1L^2 genotype would be plausible and consistent with the lower antibody level in that genotype in the B^5B^5 MHC birds. Homozygous genotypes L^1L^1 and L^2L^2 may have slower processing of antigen and longer persistence of antigen at the macrophage surface thus leading to higher antibody levels in these types.

Genotype B^2B^5 exhibited L alloantigen effects on the secondary anti-SRBC response. The L^1L^1 birds had significantly lower total and ME-resistant antibodies than

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did L^1L^2 or L^2L^2 birds. Memory B cells have higher affinity antigen receptors. The secondary responses to a T-dependent antigen produce higher affinity antibody compared with primary responses. Memory T cells respond to lower doses of antigen which suggests greater receptor efficiency. Increased numbers of reactive cells as well as altered expression of cell-surface molecules and cytokines are another secondary response characteristic (Ullman et al., 1989). The L system may affect T cells, B cells, or both populations thereby influencing the secondary response. Alloantigen L does not influence macrophage nitrite or IL-6 production (M. A. Qureshi, Department of Poultry Science, N. C. State University, Raleigh, NC 27695, personal communication). Altered production of other cytokines by macrophages or other cell types has not been examined.

Different L genotypes have distinct effects on primary versus secondary titer depending on the B genotype context. Interactions between cells bearing L and B antigens or between the antigens themselves may occur. An alternative explanation is that the L alloantigen may have a pleiotropic effect on T or B cells involved in the antibody response. Certain L genotypes (i.e. L^1L^1 and L^2L^2 in B^5B^5) may activate naïve T or B cells, or both, participating in a primary response. Other L genotypes, exemplified by L^1L^2 and L^2L^2 in B^2B^5, may mediate more efficient memory cell activation and proliferation of secondary responses.

With respect to the BA antibody response, we found no L system effect on primary antibody response but a significant effect on secondary antibody response in the B^2B^5 genotypic background. Polyclonal activation by BA may negate any response differences due to alloantigen L. The lack of observable primary effect could also be a reflection of the partial T-cell independence of the immune response to BA. Efficient

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immune responses to BA do involve both $CD4^+$ and $CD8^+$ T cells to some degree, as well as efficient isotype switching in the course of a humoral response (Golding, 2001). The L effect on secondary response to BA is only evident in a B^2B^5 background. This may result from some effects similar to those found in the secondary response to SRBC in the same background. The L system may affect cells responding to BA in the secondary response. Production of cytokines IL-1 or TNFα may be changed sufficiently to influence the secondary, but not the primary response.

Lines selected for bursa of Fabricius size had different frequencies of L alleles (Scott et al., 1988). The Small Bursa Line (SBL) had a greatly increased L^2 frequency compared with the Large Bursa Line (LBL). Primary and secondary antibodies following SRBC injection were also higher in the SBL. Immunoglobulin G was higher in the LBL (Landreth and Glick, 1973; Yamamoto and Glick, 1982). Neither bursa size nor antibody levels could be attributed to the differences in L alleles but this system may have influenced the responses. The High Antibody (HA) Line and the Low Antibody (LA) Line, selected for primary response to SRBC, were fixed for the L^2 allele (Dunnington et al., 1984; Martin et al., 1990) which precluded an assessment of an L effect in these lines. These populations are also at or near fixation for B^{21} and B^{13} in HA and LA Lines, respectively.

Precedents exist for L alleles having dissimilar effects in different B genotypes. Rous sarcoma outcome was affected by L genotype in two separate investigations. The L^1L^1 genotype had an enhanced response to Rous sarcomas compared to the L^1L^2 genotype in B^2B^5 birds (LePage et al., 2000b). Medarova et al., (2002) examined the L influence on Rous sarcomas in fully segregating combinations of two B haplotypes and

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two L alleles. The L^1L^2 genotype was associated with an enhanced response compared to the L^1L^1 and L^2L^2 genotypes in B^5B^5 birds. These two studies employed matings with different background genes and used different virus doses. Nonetheless, L genotypes produced contrasting effects in different B genotypes.

Certain similarities exist between alloantigen L and CD22, an antigen influencing mouse and human antibody responses. CD22 is an antigen of 130 to 140 kD MW expressed during functional maturation of B cells. Only two serologically-defined alleles that differ in polypetide coding sequences have been described. The two alleles vary among inbred strains of mice (Lajaunias et al., 1999). This molecule modulates the signaling threshold for B cell activation and proliferation following B cell receptor engagement (Tedder et al., 1997). Interaction of B cell CD22 occurs with other B cells, monocytes, and T cells (Stamenkovic et al., 1991). The L system has a 135 KD molecular weight and possesses two alleles as does CD22. Adding the possible L expression on white blood cells, the similarities between the two systems raise intriguing possibilities that merit further investigation.

Figure 11. Mean primary antibody titers to SRBC for L^1L^1 (n = 10), L^1L^2 (n = 16), and L^2L^2 (n = 12) genotypes within a B^5B^5 background. Bars having no common letter differ significantly ($P < 0.05$).

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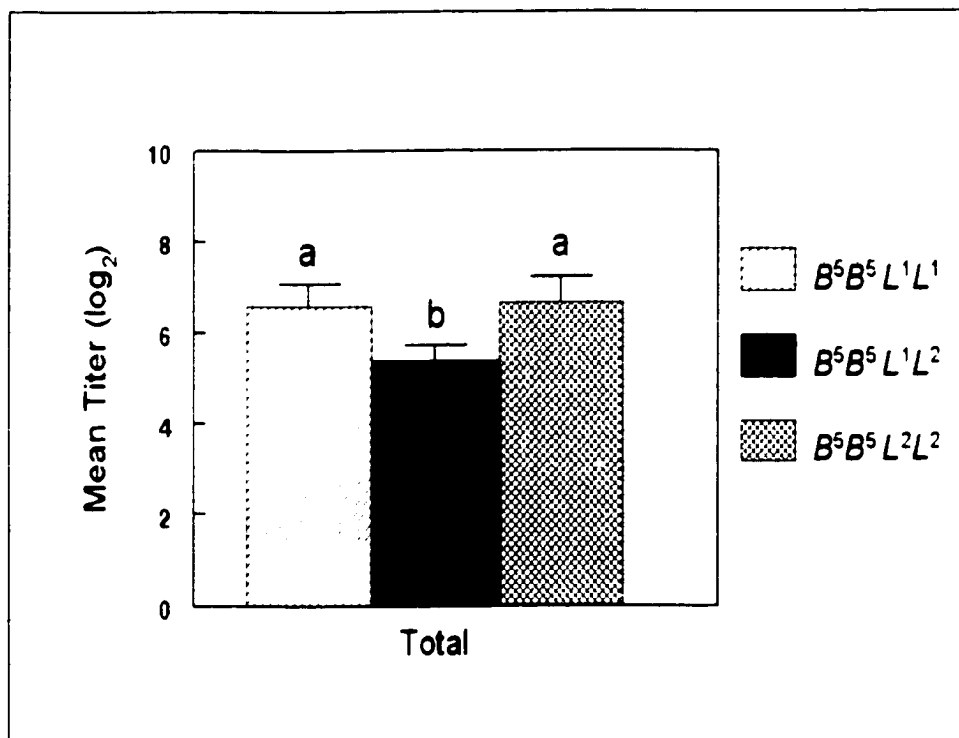
Figure 12. Mean total and ME-resistant secondary antibody titers to SRBC for L^1L^1 (n = 20), L^1L^2 (n = 48), and L^2L^2 (n = 31) genotypes within a B^2B^5 background. Bars within an antibody type having no common letter differ significantly ($P < 0.05$).
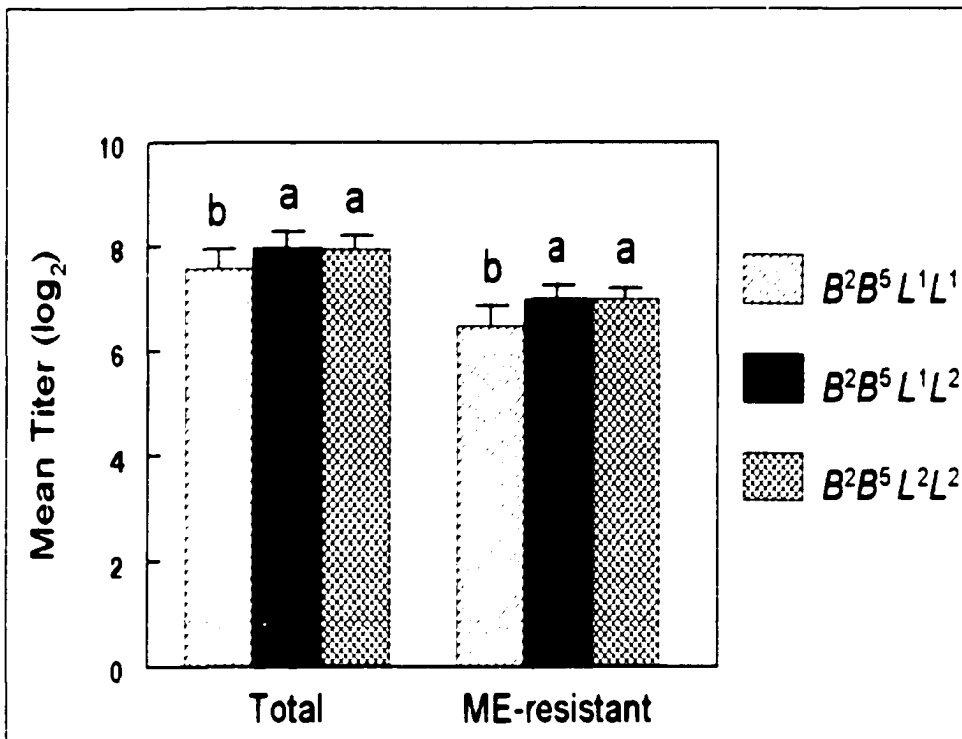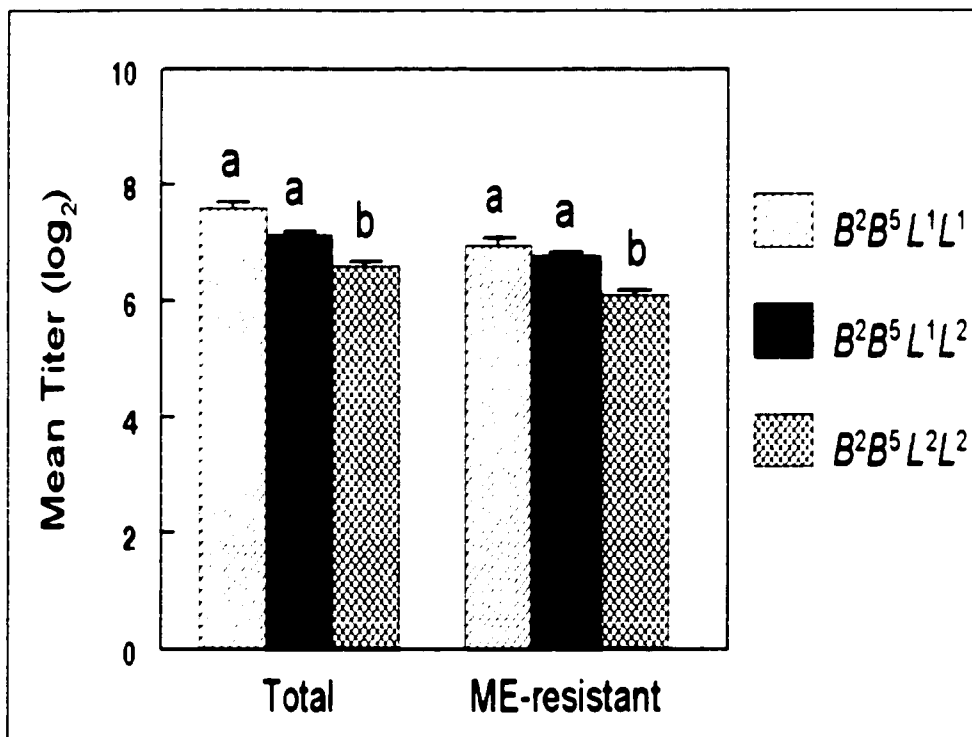
Figure 13. Mean total and ME-resistant secondary antibody titers to BA for L^1L^1 (n = 24), L^1L^2 (n = 47), and L^2L^2 (n = 29) genotypes within a B^2B^5 background. Bars within an antibody type having no common letter differ significantly ($P < 0.05$).

CHAPTER V

RESISTANCE, SUSCEPTIBILITY AND IMMUNITY TO CECAL COCCIDIOSIS: B COMPLEX AND ALLOANTIGEN SYSTEM L EFFECTS

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ABSTRACT

This study examined alloantigen system L effects on resistance and acquired immunity to *Eimeria tenella* infection in three B complex genotypes. Experimental progeny segregating for B and L genotypes were produced from pedigree matings of B^2B^5 L^1L^2 sires and dams. Chicks were weighed and inoculated with 30,000 *E. tenella* oocysts at 6 weeks of age to evaluate resistance in four trials (n = 262). Immunity was studied in four additional trials (n = 244) by immunizing progeny with 500 *E. tenella* oocysts per day for 5 days beginning at 5 weeks of age. Two weeks after the last immunization dose, the birds were weighed and challenged with 30,000 *E. tenella* oocysts. All birds were weighed again and scored for cecal lesion six days after the 30,000 oocyst dose challenge. Weight gain and cecal lesion scores were evaluated by ANOVA. Major histocompatibility (B) complex genotype did not affect resistance to *E. tenella* based on lesion score or weight gain. The B genotypes, B^5B^5 and B^2B^5, had significantly lower

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cecal lesion scores than the B^2B^2 genotype in the immune study. Weight gain was not affected. No significant L system effects with or without immunization were detected. These results are consistent with previous research demonstrating B complex effects on immunity to cecal coccidiosis. They suggest differential genetic control of immunity versus resistance. The absence of L system effects agrees with an earlier proposition that L may exercise its immune functions by controlling antibody but not cell-mediated responses .

INTRODUCTION

Cecal coccidiosis is a disease in chickens caused by the intracellular protozoan parasite, *Eimeria tenella*. The morbidity due to coccidiosis manifests itself as reduced body weight, decreased feed efficiency, and in some cases, mortality. Disease severity is judged by several criteria including weight loss, cecal lesions, fecal oocyst content, and mortality. These symptoms need to be considered simultaneously because they do not correlate. Long et al., (1980) suggested that there are at least three stages of immunity to cecal coccidiosis characterized by: 1) complete resistance to the parasite; 2) discharge of oocysts but no lesion occurrence; or 3) resistance to the clinical effects of the disease despite the presence of severe lesions.

Due to the increasing parasite resistance to the anticoccidial compounds currently used in commercial poultry production, there has been active research into the possibility of utilizing the chicken immune response as a method of disease control. One tool to counteract coccidiosis is a live vaccine consisting of low levels of all pathogenic coccidial species, which relies on repeated low dose exposures to the parasite to increase immunity (Johnson et al., 1979). Another measure that has been explored involves the

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identification of genetic factors related to immunity, which may affect the outcome of the disease. The frequency of these beneficial alleles can then be increased in commercial populations through selective breeding (Johnson and Edgar, 1982).

The B complex was one genetic region examined for its effects (Ruff and Bacon, 1989; Clare et al., 1985). Ruff and Bacon (1989) found that the B^2B^2 congenic Lines 15.6-2 and 15.7-2 had greater susceptibility to 10^5 *Eimeria acervulina* oocysts than congenic Line 15.151-5 (B^5B^5). These lines had similar susceptibility to *E. tenella* infection. The authors suggested that although the B complex influenced resistance and susceptibility to *E. acervulina* coccidiosis, these genes might have only a minor role in immunity to challenge infection. Their results pointed to a difference in the genes controlling initial infections and the genes controlling immunity to subsequent challenges. B complex effects on immunity also differed with *Eimeria* species. After repeated low dose immunization, Clare et al. (1985) observed that B^2B^2 birds had lower immune protection against *E. tenella* infection than the B^5B^5 genotype, whereas Ruff and Bacon (1989) found lower immunity in congenic Line 15.151-5 B^5B^5 than in congenic Lines 15.6-2 and 15.7-2 (B^2B^2).

The differences between the two studies with regard to the effect of the B system on disease immunity were attributed to the different immunization protocols and different genetic backgrounds. In support of this, Ruff and Bacon (1989) demonstrated that B^2B^2 congenic lines had low immunity to cecal coccidiosis following a single 100-oocyst immunization but a high degree of immunity following four 100-oocyst immunizations. Furthermore, Clare et al. (1985) found evidence that the response to coccidia varied among genotypes depending on the form of the challenging antigen. The B^5B^5 but not

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B^2B^2 and B^2B^5 birds developed a delayed wattle reaction to a nonviable oocyst antigen. Both B^5B^5 and B^2B^5 chickens had a greater response to viable asexual stages than did the B^2B^2 genotype. This result suggested that the immune response to the disease might reflect both the antigen presentation mechanism and the particular character of parasite antigens presented by the MHC.

The absence of correlation between B haplotype effect on resistance to cecal coccidiosis and immunity to the disease pointed to genes other than the MHC as potential factors influencing the response to *E. tenella* infection. Lillehoj et al. (1989) found wide variations in innate resistance and acquired immunity to cecal coccidiosis between birds sharing a common genetic background but differing at their MHC as well as between birds sharing a common B haplotype but differing with respect to their genetic backgrounds. Other studies identified some of the nonMHC genes, including the *A-E* (Johnson and Edgar, 1984), *I* (Martin et al., 1986), and *C* (Johnson and Edgar, 1986) alloantigen systems.

A study on resistance and susceptibility to *E. tenella* as a function of eight alloantigen systems found a significant L system effect on lesion scores within a defined MHC background (Taylor and Briles, 2000). $B^2B^2L^1L^1$ chickens had higher lesion scores than $B^2B^2L^1L^2$ and $B^2B^2L^2L^2$ chickens. No L effects were evident in a B^2B^5 genotypic background. Alloantigen system L has demonstrated effects on immune functions including monocyte phagocytosis (Qureshi et al., 2000), antibody response to sheep red blood cells and *Brucella abortus* (Medarova et al., unpublished data), and bursa size (Scott et al., 1988). Two studies have reported a significant L effect on Rous sarcoma

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outcome (LePage et al., 2000b; Medarova et al., 2002). DeSilva (1965) also found fertility was influenced by the L system.

We sought to characterize alloantigen L effects on resistance, susceptibility, and acquired immunity to cecal coccidiosis in chickens having fully segregating combinations of two B and two L alleles. The chickens were produced by the same mating types used to study the L system's role in Rous sarcoma outcome (Medarova et al., 2002) as well as antibody responses to SRBC and *Brucella abortus* (Medarova et al., 2002, unpublished data). These matings minimize the influence of other background genes, reveal possible interrelationships between L and B systems, and facilitate discrimination between factors affecting resistance to *E. tenella* infection and factors affecting immunity to the disease.

MATERIALS AND METHODS

Stock. Modified Wisconsin Line 3 x Line NIU 4 sires ($B^2B^2L^1L^2$) were crossed to inbred Line 6.15-5 dams ($B^5B^5L^1L^1$) to produce the parental stock consisting of 50% inbred Line 6.15-5 with the $B^2B^5L^1L^2$ genotype (Medarova et al., 2002). Pedigree matings of 4 B^2B^5 L^1L^2 sires to 5 B^2B^5 L^1L^2 dams per sire, produced experimental progeny that segregated for all possible combinations of B and L alleles. The chicks were hatched at the University of New Hampshire Poultry Research Farm and were wing-banded for identification. Vaccinations against Marek's disease and Newcastle-bronchitis were administered at hatch and 10 d, respectively. The chicks were housed in isolation, free from coccidial exposure, in wire floor cages with free access to antibiotic-free water and feed.

Alloantigen typing. The chickens were typed for B and L systems in agglutination assays utilizing antisera specific for the haplotypes of the parental stocks

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(Briles and Briles, 1982) as described (LePage et al., 2000b). After the chicks reached 3 wk of age, 0.5 ml blood was drawn from the wing vein into cold sodium citrate anticoagulant solution (68 μM sodium citrate / 72 μM sodium chloride). Samples were shipped overnight with ice packs to Northern Illinois University. Fifty μl of a 2% suspension of washed red blood cells was dispensed into tubes containing 100μl of antiserum specific for the B and L system haplotypes of interest. Following a 2-hr room temperature incubation, the reaction mixtures were transferred to 3°C for an overnight incubation. The following day, cells were resuspended and scored visually for agglutination after a 1-hr incubation at room temperature.

Coccidial Cultures. Cultures of the Lilly 65 strain of $E.$ $tenella$ oocysts were obtained from a stock culture stored at the University of New Hampshire. In $vivo$ propagation of the stock culture involved inoculation of 3- to 5-wk-old birds with 50,000 sporulated oocysts per bird. Seven d post-inoculation, oocysts were harvested from the cecal pouches. The cecal contents were subjected to peptic digestion (Rikimaru et al., 1961). Oocyst sporulation was induced by bubbling with air in 0.5% potassium dichromate at room temperature. The sporulated oocyst mix was sterilized in a 50% chlorine bleach solution (Wagenbach and Burns, 1969). Inocula were counted using a hemocytometer and administered per os to the crop using an inoculation tube and a syringe.

Resistance and Susceptibility to E. tenella. Four hatches having 262 experimental progeny were used to evaluate resistance and susceptibility to $E.$ $tenella$. Six-week old birds were weighed and inoculated with a single dose of 30,000 $E.$ $tenella$ oocysts. The birds were weighed again and cecal lesions were scored six d after

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challenge. The inoculated birds were compared to an unchallenged control group (n = 32) from the same parental matings that produced the experimental chicks.

Immunity to E. tenella. Progeny from 4 hatches having two hundred and forty four chicks were used to study immunity to *E. tenella*. Birds were immunized with 500 *E. tenella* oocysts per day for 5 days beginning at 5 weeks of age. Two weeks after the last immunization dose, the birds were weighed and challenged with 30,000 *E. tenella* oocysts. Six days after challenge, the birds were weighed again and cecal lesion scores were assessed. The immunized birds were compared to uninfected control (n = 12) and unimmunized, challenged (n = 27) control groups consisting of birds from the same parental matings.

Evaluation Criteria. Cecal lesion scoring followed the procedure outlined by Johnson and Reid (1970), where 0 = no gross lesions; 1 = very few scattered petechiae on the cecal wall, no thickening of the cecal walls, normal cecal contents; 2 = lesions more numerous with noticeable blood in the cecal contents, cecal wall somewhat thickened, normal cecal contents; 3 = large amounts of blood or cecal cores present, cecal walls greatly thickened, little or no fecal contents in the ceca; and 4 = cecal wall greatly distended with blood or large caseous cores, fecal debris lacking or included in cores. Dead birds were given a score of 4. Weight gain was calculated by subtracting the initial weight obtained at challenge from the weight obtained 6 d following challenge.

Statistical Analysis. Weight gain and mean cecal lesion scores were analyzed by least squares ANOVA with hatch, sex, sire, dams within sires, *B* genotype, *L* genotype, and *B* x *L* interaction as main effects. This procedure was used in both the resistance and susceptibility study and the immune study. In an additional analysis for immunity, weight

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gain and cecal lesion scores of immunized birds were compared with the unimmunized, challenged group to assess the efficacy of the immunization protocol. Significant means were separated by Fisher's protected LSD at $p < 0.05$.

RESULTS

Ea-B and *Ea-L* segregated independently of each other in the experimental progeny. Neither the *B* nor the *L* system significantly affected weight gain or lesion score in the resistance and susceptibility study. The B^2B^2 genotype had the numerically highest cecal lesion scores (3.00 ± 0.09) whereas the B^5B^5 genotype had the lowest lesion scores (2.74 ± 0.10). However, these differences were not statistically significant. The L^1L^1, L^1L^2, and L^2L^2 genotypes had cecal lesion scores of 2.85 ± 0.10, 2.83 ± 0.08, and 2.84 ± 0.10, respectively, that were not statistically different (Fig. 14).

The B^2B^2 birds that had the highest lesion scores also had the lowest weight gain (26.49 ± 6.44 g) and the B^5B^5 birds had the lowest lesion scores but the highest weight gain (32.16 ± 4.73 g) [Fig. 15]. These cecal lesion scores and weight gains were negatively correlated (correlation coefficient = -0.989). Weight gain did not differ significantly among the three *L* genotypes. The lowest weight gain (27.29 ± 5.66 g) occurred in L^1L^1 birds and the highest weight gain (31.75 ± 3.81 g) was found in the L^1L^2 birds (Fig. 15).

In the immune study, significant differences in cecal lesion score ($p < 1 \times 10^{-6}$) [Fig. 16] and weight gain ($p < 1 \times 10^{-4}$) were found between the immune group and the unimmunized, challenge control group. The unimmunized, unchallenged control group had no cecal lesions. Together, these data indicated that the immunization protocol used

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was successful. The B genotype had a significant effect on lesion score (p < 0.007) in immunized progeny. The B^2B^2 genotype had significantly higher lesion scores (1.61 ± 0.14) than either the B^2B^5 (1.21 ± 0.09) or B^5B^5 (1.00 ± 0.14) genotypes (Fig. 16). Weight gain was not affected significantly by B genotype (data not shown) as the B^2B^2 birds gained 22.80 ± 4.12 g and the B^5B^5 birds gained 30.74 ± 4.72 g. The correlation coefficient between weight gain and lesion scores as a function of B genotype was lower than in the resistance and susceptibility study (correlation coefficient = -0.561).

L genotype had no significant effect on lesion scores or weight gain (data not shown). There were no significant lesion score differences among the L^1L^1 (1.23 ± 0.15), L^1L^2 (1.29 ± 0.09), and L^2L^2 (1.25 ± 0.13) genotypes. The weight gains according to the L genotypes were L^1L^1 (24.87 ± 4.12), L^1L^2 (21.95 ± 2.95), and L^2L^2 (23.59 ± 3.81), respectively. These values did not differ significantly.

DISCUSSION

The current study found no B or L system effects on resistance and susceptibility to *E. tenella*. Responses to the parasite varied according to B genotype in UCD B complex congenic chickens (Caron et al., 1997). Genotype B^2B^2 had significantly higher lesion scores than B^3B^3, $B^{18}B^{18}$, and $B^{21}B^{21}$ birds after oocyst challenge. Ruff and Bacon (1989) found that neither weight gain nor lesion score differed among 15.B congenic lines 15.6-2 (B^2B^2), 15.7-2 (B^2B^2), and 15.151-5 (B^5B^5). Lillehoj et al., (1989), showed no significant differences in *E. tenella* lesion scores in seven 15.B congenic lines. On the other hand, Line SC B^2B^2 chickens produced fewer oocysts than both congenic 15.6-2 (B^2B^2) and 15.7-2 (B^2B^2). The genetic background differences of the three B^2B^2 lines suggested a role for nonMHC genes in resistance and susceptibility to cecal coccidiosis.

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Our results on the B complex effect on immunity to $E.$ $tenella$ agree with previous findings (Clare et al., 1985; Caron et al., 1997). B^2B^5 and B^5B^5 birds had significantly lower lesion scores than the B^2B^2 birds but the three genotypes did not differ in terms of weight gain (Clare et al., 1985). Repeated low dose immunization followed by 10,000 oocyst challenge in UCD B congenic lines revealed lower lesion scores in B^3B^3 and B^QB^Q chickens compared with the $B^{19}B^{19}$, $B^{24}B^{24}$, $B^{14}B^{14}$, and B^2B^2 genotypes (Caron et al., 1997). The lower immune response of B^5B^5 chickens compared with B^2B^2 birds in immunity experiments by Ruff and Bacon (1989) did not agree with these three studies. Differences in genetic backgrounds, immunization protocols, and parasite strains are three factors that possibily influenced the variabilty in B complex effects.

Clare et al. (1989) studied resistance, susceptibility, and immunity to $E.$ $tenella$ in B-F/B-G recombinant birds to identify the MHC regions contributing to differential resistance and immunity. The B-F region significantly influenced disease susceptibility. Furthermore, the results supported the concept that different genetic mechanisms influenced innate resistance to cecal coccidiosis compared with acquired immunity. Although B-F^2 birds showed greater resistance to the disease, they demonstrated little protection after priming with the antigen. Conversely, B-F^{21} birds, while being susceptible to initial infection with the parasite, displayed enhanced protection following immunization.

Cell-mediated responses play a central role in the induction of protective immunity against $E.$ $tenella$. Antibody responses contribute only marginally to protection. Genetic variation in humoral responses following infection with $Eimeria$ has not been observed, while such variation in T-cell responses has been demonstrated conclusively

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(Lillehoj and Trout, 1993; Bumstead et al., 1995). The disparate effects of B haplotype on resistance versus immunity could be a reflection of the differential contribution of T cell subsets to the two processes. While CD4$^+$ T cells have been implicated for their role during primary infection, CD8$^+$ T cells have been defined as key components during secondary infection (Trout and Lillehoj, 1996, Lillehoj, 1998). In addition, CD8$^+$ T lymphocytes have been proven important as transporters of sporozoites to the epithelial sites where they develop, further complicating the understanding of these cells' contribution to the anti-coccidial immune response (Trout and Lillehoj, 1993). *Eimeria* parasites are characterized by complex life cycles, with each stage expressing different surface antigens. The complex life cycle means that the parasite immunogenicity changes through the course of an infection increasing the difficulty of determining the mechanisms behind the anti-coccidial immune response (Lillehoj et al., 1989).

It is plausible that certain MHC haplotypes would favor responses defined predominantly by CD8$^+$ T cell subpopulations, whereas other haplotypes would be associated with enhanced CD4$^+$ T-cell activity. Different MHC haplotypes would vary in the recognition efficiency for antigens defining specific parasite life cycle stages, meaning that particular B complex haplotypes would differ in their contribution to primary versus secondary immunity. Furthermore, certain MHC haplotypes are conducive to better innate resistance whereas others mediate better acquired immunity (Clare et al., 1989).

Divergent selection for resistance and susceptibility to *E. tenella* infection altered MHC and nonMHC alloantigen frequencies (Johnson and Edgar, 1984; 1986). The B^5 and B^r, a recombinant, haplotypes were unique to the resistant line. Haplotypes B^1, B^3, B^4,

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and B^6 were exclusive to the susceptible line. The B^2 haplotype occurred in both lines but at a higher frequency in the resistant line (Johnson and Edgar, 1986). The selected groups had significantly different allele frequencies at the A-E and C loci. A high proportion of the resistant birds had the A^7E^5 and A^9E^1 genotypes but the majority of the susceptibile birds had the A^9E^3, A^9E^5, and A^9E^2 genotypes (Johnson and Edgar, 1984). The resistant line carried the C^4 allele, while the susceptible line carried the C^2 and C^3 alleles (Johnson and Edgar, 1986).

Martin et al. (1986) examined the effect of alloantigen systems segregating in lines selected for antibody response to sheep erythrocytes on natural and controlled exposures to cecal coccidiosis. Their results showed an alloantigen I effect on lesion scores in the high antibody responder line, HA, that had the $B^{21}B^{21}$ genotype. Birds derived from a mating between I^4 homozygous parents had significantly greater resistance than birds derived from I^2I^4 x I^4I^4 and I^2I^4 x I^2I^4 matings.

Evidence for nonMHC genetic effects on coccidiosis suggested that alloantigens might contribute to these effects. In particular, alloantigen L affected phagocytosis (Qureshi et al., 2000), antibody responses (Medarova et al., unpublished data), and Rous sarcoma outcome (LePage et al., 2000b; Medarova et al., 2002). The L alloantigen is polymorphic with two segregating alleles, L^1 and L^2, (Gilmour, 1959; Briles, 1962) that encode proteins of approximately 135 kD (Ameiss and Briles, 2000). The L proteins are found on chicken erythrocytes as well as leukocytes (Kopti and Briles, unpublished data). Gilmour (1959) studied blood group polymorphisms following 18 generations of full-sib matings from an outbred line. Segregation was maintained at only three loci, including L,

which suggested a possible survival advantage associated with the preservation of heterozygosity.

Taylor and Briles (2000) found a significant L system effect on resistance to $E.$ *tenella* measured by lesion scores in a B^2B^2 MHC background (Taylor and Briles, 2000). The experimental chickens were 50% Wisconsin Line 3 and 50% Line NIU 4 White Leghorns. In the current study, no L effect was observed in birds with a different genetic composition of 50% Line 6.15-5, 25% Wisconsin Line 3 and 25% Line NIU 4 White Leghorns. This group also had full segregation for the B and L alleles. The dissimilar genetic backgrounds must have contributed to the different results in the two experiments. The lack of observable L system effects on either resistance or acquired immunity to $E.$ *tenella* infection in the chickens of this study means that this alloantigen did not influence cells that generate responses to the parasite.

The negative correlation between weight gain and lesion score as a function of B genotype was high (coefficient = - 0.989) in the resistance and susceptibility study. The same correlation was much lower (coefficient = - 0.561) in the immune study. Neither study found an effect of B or L genotype on weight gain. Previous studies have also found a low correlation between weight gain and lesion scores in studies of immunity to $E.$ *tenella* (Long et al., 1980; Clare et al., 1985; Martin et al., 1986; Caron et al., 1997).

In conclusion, consistent with a previous study (Clare et al., 1985), the B complex affects immunity but not resistance and susceptibility to cecal coccidiosis. These effects could be due to differential genetic mechanisms involved in acquired versus innate immunity to the parasite through dissimilar contributions of different MHC haplotypes.

The *L* system did not affect either innate resistance or acquired immunity to the disease in this study. However, these results do not rule out potential effects of other nonMHC loci.

Figure 14. Mean (± SEM) cecal lesion scores for segregating B and L genotypes six d after 30,000 *Eimeria tenella* oocyst challenge at six wk of age. Numbers of birds for each genotype were $B^2B^2 = 67$, $B^2B^5 = 121$, $B^5B^5 = 74$, $L^1L^1 = 72$, $L^1L^2 = 126$, and $L^2L^2 = 64$. NS = not significant.
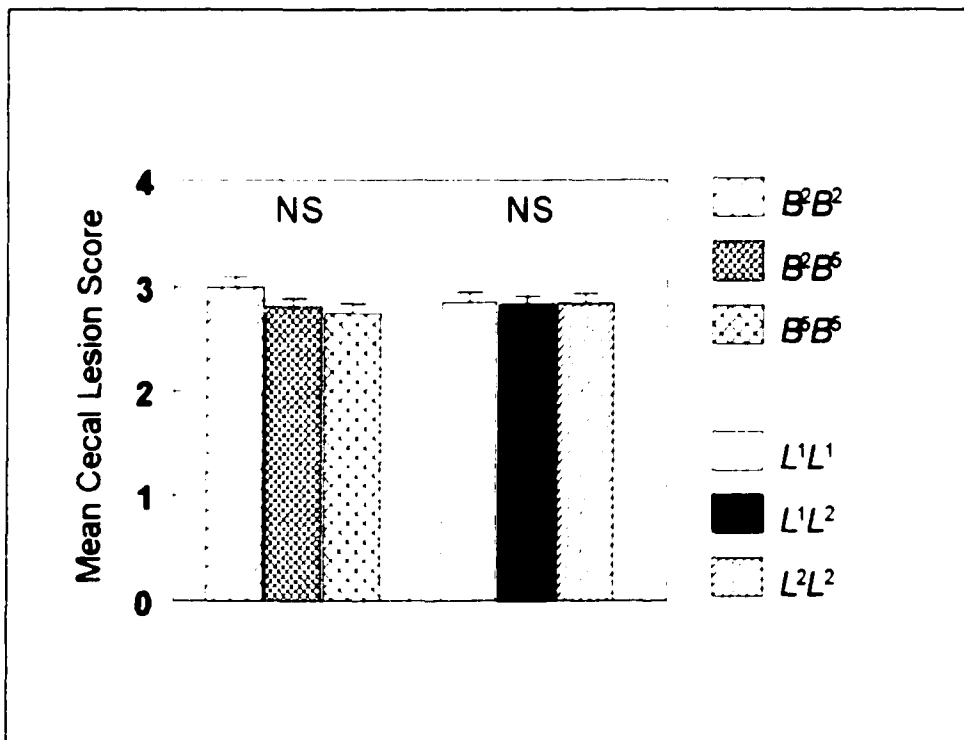
Figure 15. Mean (± SEM) weight gain (g) for segregating B and L genotypes six d after 30,000 *Eimeria tenella* oocyst challenge at six wk of age. Numbers of birds for each genotype were B^2B^2 = 67, B^2B^5 = 121, B^5B^5 = 74, L^1L^1 = 72, L^1L^2 = 126, and L^2L^2 = 64. NS = not significant.
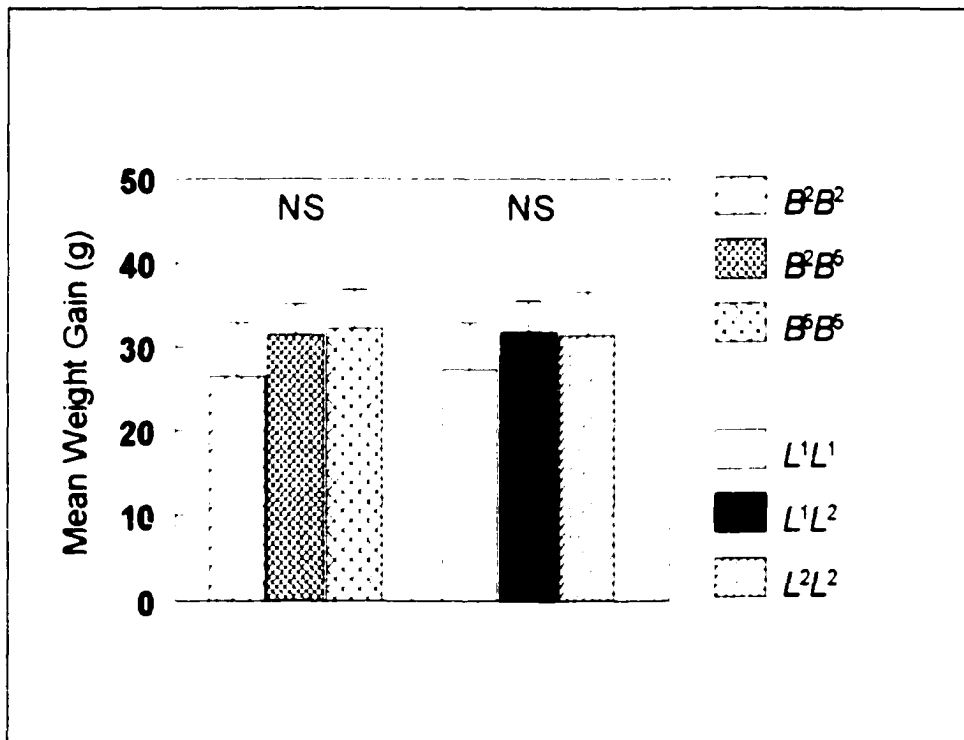
Figure 16. Mean (± SEM) cecal lesion scores for segregating B genotypes immunized with 500 *Eimeria tenella* oocysts for 5 consecutive d at five wk of age followed by challenge with 30,000 *Eimeria tenella* oocyst 14 days after the last immunizing dose. Numbers of birds for each genotype were B^2B^2 = 66, B^2B^5 = 131, B^5B^5 = 47. Uninoculated controls (n = 12) were not exposed to oocysts whereas the challenge control birds (n = 27) received the challenge only. Bars in the B genotype that have no common letter differ significantly.
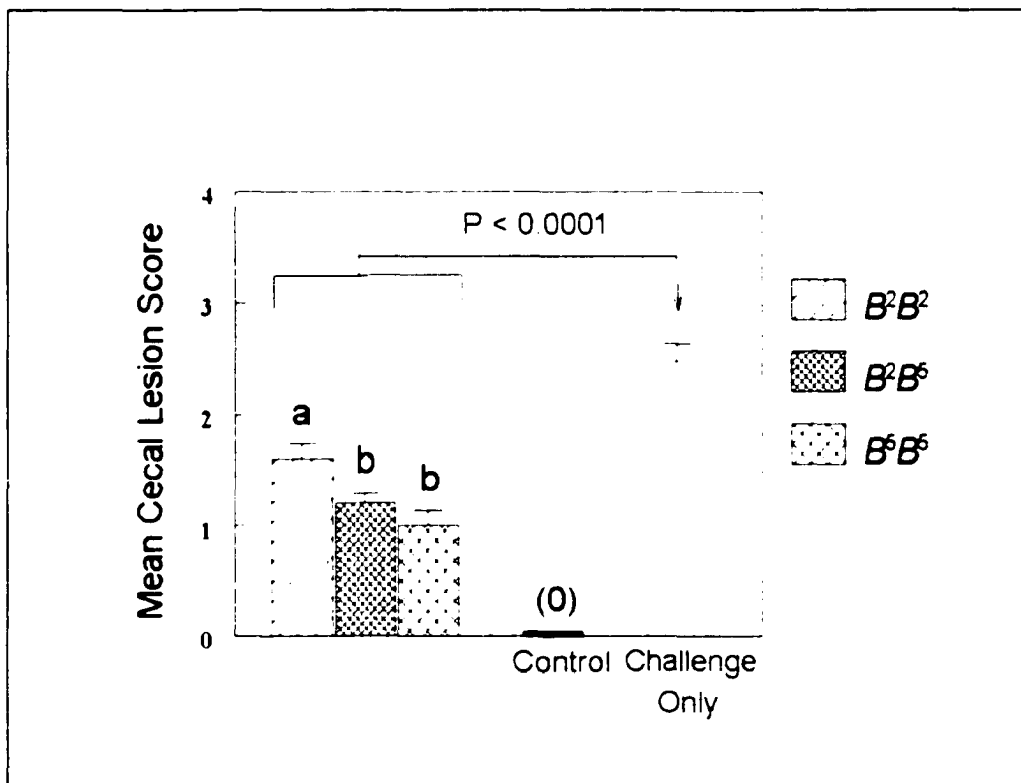
CHAPTER VI

CONCLUSIONS

We studied the effect of erythrocyte alloantigen L on the outcome of Rous sarcomas, on antibody response to sheep erythrocytes (a T-dependent antigen) and *Brucella abortus* (a partially T-independent antigen), as well as on innate resistance and acquired immunity to *Eimeria tenella*. We found that $Ea-L$ affects Rous sarcoma tumor fate and antibody response to sheep red blood cells and *Brucella abortus*, but not the outcome of *E. tenella* infection. Furthermore, we observed $Ea-L$ effects only within certain major histocompatibility (B) complex genotypic backgrounds, suggesting a co-dependence between the effects of the two systems, whether as a result of a physical or a purely functional interaction.

Based on the fact that $Ea-L$ affects antibody response to sheep erythrocytes and *Brucella abortus*, and does not affect the outcome of cecal coccidiosis, which is defined primarily by genetic variation at the cell-mediated level, we tentatively propose that alloantigen L exercises its functions by controlling humoral immunity. The apparent effect of $Ea-L$ on Rous sarcomas could represent evidence of the participation of antibody-mediated mechanisms in the determination of virally-induced-tumor fate.

Together with the current knowledge about the molecular characteristics of $Ea-L$, i.e., its molecular size (135 kD), its polymorphism (the presence of two serologically distinguishable alleles), and its potential expression on leukocytes, our findings point

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toward a possible similarity between *Ea-L* and the mammalian CD22 antigen, expressed on B lymphocytes and controlling the signaling threshold through the B cell receptor.

Currently, we have identified a clone in a chicken EST library, which displays significant homology to the mammalian *Cd22* gene. Partial sequencing of the purified L antigen and comparison of the resulting sequence to that clone as well as to sequences deposited in various gene and protein sequence databases would provide more conclusive evidence as to whether *Ea-L* is indeed the avian homologue of CD22.

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