INTRODUCTION

The major histocompatibility complex (MHC) is a cluster of genes present in all vertebrate species, including man. This gene complex controls a variety of critical immune functions.

The MHC in mice is located on chromosome 17 and includes about 4000 kb of DNA. This segment of the DNA encodes three different classes of proteins: class I and class II which are integral cell-surface glycoproteins, and class III which encode serum components of the complement system. The class I molecules are the classical transplantation antigens (encoded by the K, D, and L regions of the MHC) and influence the behavior of the cytotoxic T lymphocytes, whereas the class II antigens are the La antigens which regulate immune recognition and antigen presentation by B cells, T cells and macrophages. The I region of the MHC which encodes the La antigens, is further divided into I-A and I-E. The class I and class II genes are highly polymorphic. It is estimated that over 50 alleles exist for the K, D, Aα, Aβ and Eβ genes.

This paper will attempt to review the early literature regarding the MHC of the mouse and explore their functional significance as it relates to immune regulation and induction of disease.

HISTORICAL PERSPECTIVE

The study of the MHC in the mouse model was aided by the existence of inbred strains of mice produced by mouse fanciers for commercial purposes. In 1903, Carl Jensen discovered that a tumor arising in a closed-bred albino mouse stock could be transplanted through 19 generations of mice from the same stock. This tumor when implanted to another strain, was rapidly rejected. Little and Tyzzer (1916) further studied this phenomenon and formulated a genetic theory for tumor transplantation. He proposed that susceptibility to a tumor transplant was governed by several dominant genes. They further confirmed this finding by developing sublines within a strain that was resistant to tumors growing in the original strain.

Gorer, in a paper published in 1936, studied red blood cell transplantation antigens in three different inbred strains of mice. He immunized rabbits with blood from each of these strains and then performed a series of absorption and agglutination studies with the antisera and red blood cells of the different strains. From a wide panel of experiments, the results indicated the presence of four blood group antigens which he referred to as antigenic factors I, II, III and IV. The antigenic factor II was later shown by Gorer to also be present on tumor cells. Gorer, in 1938, thus formulated an immunologic theory of tumor transplantation - "Normal and neoplastic tissues contain isoantigenic factors (alloantigens) which are genetically determined - Isoantigenic factors present
in the grafted tissue and absent in the host are capable of eliciting a response of the graft". This immunologic theory confirmed an earlier theory proposed by Haldane (1933) who also believed that tumors were rejected because of the presence of foreign alloantigens on their surface.

The first concrete evidence for the involvement of the immune response was reported by Gibson and Medawar in 1943. They obtained visual and histological evidence and very accurately described the fate of primary and secondary human skin auto and allografts. Their conclusion was that graft rejection was mediated by antibodies. Medawar, the following year published another paper describing the behavior and fate of skin allografts in rabbits and confirmed his earlier conclusion. In this study, graft rejection was shown to be dose dependent, specific and immune mediated. Mitchison in 1954 studied the growth patterns of a lymphosarcoma in resistant and susceptible mice. In an attempt to transfer immunity to tumors, he showed that grafts of the lymph nodes draining the site of tumor implantation were the only successful means of conferring immunity. Serum, peritoneal exudates and lymphocyte suspensions had no effect. These experiments indicated that transplantation antigens induce a cell-mediated immune response. Snell in 1948 termed the antigens responsible for tissue compatibility, histocompatibility antigens and the genes encoding for these antigens histocompatibility genes or H genes. Snell further continued his studies by developing what he called isogenic resistant strains of mice. Isogenic (congenic) mice are inbred strains of mice that are genetically identical except for some highly restricted genetic region; the H-2 region. Using this congenic strain of mice, Snell and Gorer established the identity of the gene for tumor resistance with the gene coding for antigen II. This histocompatibility gene was termed H-2 and was found to be linked to a gene for fused tail located on chromosome 17.

Benacerraf and his co-workers (Levine et al. 1963) first identified the immune response (Ir) genes using the synthetic polypeptide poly-L-lysine (PLL) as an immunogen. Two inbred strains of guinea pigs were used, strain 2 which could respond to DNP-Poly-L-lysine and strain 13 which could not. A cross between these two strains, however, produced responder F1 animals. This work led to the use of other synthetic polymers such as poly-L-arginine, random copolymers of L-glutamine and L-alanine (GA), L-glutamic acid and L-tyrosine (GT), and L-glutamic acid and L-lysine (GL) (Bluestein et al. 1971). Strain 2 guinea pigs are high responders to PLL and GA while strain 13 is a high responder to GT. Using randomly bred Hartley guinea pigs, the genes regulating responsiveness to GA and PLL were shown to be linked. A small population of these guinea pigs, however, responded to GA but not to PLL and vice versa. Thus, the response to GA and PLL could be separated as a result of a crossover, indicating presence of two separate genes. Using similar experimentations in the mouse model, McDevitt and his co-workers (McDevitt et al. 1972) mapped the Ir genes within the mouse H-2 complex and this was designated the I region.

STRUCTURE OF THE MHC OF THE MOUSE

The MHC of the mouse comprises three classes of genes and molecules. Class I products are expressed on membranes of all nucleated cells, class II products are expressed on the membranes of lymphoid cells and monocytes, and class III products determine the structures of serum complement components. The figures below show the genes of the MHC and products of the MHC.
PRODUCTS OF H-2 LOCI

Chromosome 17

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Polypeptide chain

Membrane

Chromosome 2

\(\alpha_2\)-microglobulin
1. Class I Products.

The class I products encoded in the H-2K,D and L regions of the mouse and HL-A, B and C of humans are the classical transplantation antigens. These molecules were the first MHC products to be defined through their role in graft rejection and serologically through their induction of specific antibodies in incompatible recipients. The class I molecules are integral cell surface glycoproteins composed of two polypeptide chains, a heavy and a light chain. The heavy chain is about 45,000 daltons in molecular weight and is encoded by the MHC. The light chain (12,000 daltons) is the β2 microglobulin and is encoded by a gene located on chromosome 2 in the mouse. The heavy chain has been divided into five domains. The extracellular domains, α1, α2, α3 are each about 46 amino acid residues in size; the other two domains (about 40 residues) are the membrane spanning and cytoplasmic tail. β2-microglobulin interacts non-covalently with the α3 domain. Carbohydrate side chains are also present in the heavy chain α and α2 domains and occasionally in the α3 domain.

2. Class II Products.

The class II antigens or Ia antigens (DR in humans) are products of the genes mapping in the I region. They are present on B lymphocytes, macrophages, activated T cells, dendritic cells, Langerhan cells and epithelial cells. The class II molecules play a part in antigen recognition and cell-cell interactions in the immune response. The class II molecules are encoded by the I-A and I-E subregions of the mouse MHC. The two biochemically characterized Ia antigens are heterodimers composed of a 33,000 and 35,000 molecular weight α chain and 28,000 to 31,000 molecular weight β chain. The molecular weight differences
between the α and β chains is a result of glycosylation: two carbohydrate side chains are attached to the α chain and one to the β chain. The overall structure of the I-A and I-E antigens can be divided into an extracellular domain, a transmembrane domain and a cytoplasmic membrane. The α chain consists of two extracellular domains, α1 and α2 (90 amino acids). The α1 domain is the N-terminal domain and lacks a disulfide loop whereas the α2 domain contains a disulfide loop. The β chains also consist of two domains of similar size; β1 and β2. Each of the β domains contain a disulfide loop. The transmembrane portion of both the α and β chain is composed of about 25 hydrophobic amino acids which are followed by the intracellular region which is about 15 amino acid residues in length.

3. Class III Antigens.

The H-2 region also encodes genes for complement component C4. The C4 proteins have a molecular weight of 208,000 daltons and consists of three covalently linked polypeptide chains, α chain (87,000), β chain (78,000) and γ chain (33,000). This region designated as the 'S Region', also encodes genes for other complement components (C2 and Bf) and a gene for the cytochrome 450P proteins which is involved in the synthesis of 21 hydroxylase enzyme.

4. Other MHC Products/Regions.

In the mouse, many other genes also exist that are outside the MHC but are of interest. These genes encode molecules that are expressed on the surface of thymocytes (Tla) and on subsets of T cells (Qa). The functional relationship of these antigens are not clear.
MHC POLYMORPHISM

One of the most unique characteristics of the MHC genes are the extensive polymorphism. Many of the MHC genes are known to have anywhere between 50 and 100 alleles. A composite of all the MHC genes within an individual strain is designated as its haplotype. Thus, with polymorphism within each MHC gene, there could be millions of haplotypes in the wild population. There are very few gene systems in the mammalian genome with such polymorphism. This obviously is advantageous to the species in surviving against an onslaught of millions of infectious and disease-causing antigens. This polymorphism is maintained by four mechanisms.

1. **Mutation.**

A high rate of mutations seem to occur in the non-expressed MHC genes (pseudogenes) since there is no selection pressure involved for these genes. Thus, these point mutations accumulate in these genes. Occasional mutations also occur in the expressed MHC genes (K, D, L, A, E).

2. **Gene Conversion.**

Periodically, there is a transfer of mutated segments of these pseudogenes (Qa, Tla) to the expressed genes. The exact mechanism by which this gene conversion occurs is still not clear. Since gene conversion occurs between genes separated by a considerable distance on the chromosome, some kind of transposable elements are probably involved. Further, gene conversions seem to occur primarily between exons rather than introns. Thus, a class I or class II gene periodically receives segments of new sequences which completely change their three dimensional properties. This type of gene conversion increases polymorphism but at the same time keeps interspecies divergence to a minimum.

3. **Recombinational 'Hot Spots'.**

The MHC chromosome contains several recombinational hot spots. Crossing-over occurs primarily at these hot spots. Thus, gene distances within MHC genes cannot be determined on the basis of crossing over frequency since crossing-over would occur between genes which are very closely linked. These hot spots do not occur at the same chromosomal location in every haplotype. This suggests that these hot spots are sequence specific. These recombinants create new combinations of MHC genes generating new haplotypes.

4. **Unequal Crossing-Over.**

The MHC complex seem to be in a constant state of expansion and contraction. Some haplotypes have 30 class I genes while other only 10. The main way this occurs is by unequal cross-overs which lead to duplications and deletions. These unequal crossovers seem to be much less frequent and an exact mechanism or cause are still unclear.

FUNCTIONAL ROLE OF MHC MOLECULES

MHC antigens play a central role in the development of immune responses. Cytotoxic T cells need to recognize antigens in the context of class I molecules. Helper T cells on the other hand, respond to antigens in association with class II molecules.
1. Class I Molecules.

Cell-mediated immunity is the primary defense against diseases caused by intracellular parasites such as viruses, and also against tumors. Zinkernagel and Doherty (1979), using a virus-specific cytotoxic assay, have demonstrated that cytotoxic T lymphocytes (CTL) could lyse virus-pulsed target cells only if both the virus-specific CTL and the target cell share the same class I molecule. That is, a CTL will recognize an antigen only if the antigen is presented to the CTL in conjunction with the same class I molecule as the primary challenge. This process termed 'H-2 restriction' has been shown to be true for many viruses, haptenic groups and for minor transplantation antigens (Shearer, 1975 and Bevan, 1979). In the case of CTL, the restricting element has been localized to the H-2K and H-2D regions of the MHC.

2. Class II Molecules.

Cooperation between lymphocytes is also restricted to the class II products. Helper T cells which interact with B cells to provide the signal for the B cells to produce antibodies will only do so if the antigen is presented to them on the surface of antigen presenting cells (macrophages) which are syngeneic at the I-A subregion. The helper T cell will not respond if the antigen is presented with a different class II molecule. This class II restriction has also been demonstrated for delayed-type hypersensitivity reactions (Miller et al. 1979). Transfer of DTH can only be effected if donor and recipient share class II products.

T CELL ANTIGEN/MHC RECEPTOR

Investigators spent years in the pursuit of defining and characterizing T cell receptors on the surface of T cells which recognize foreign antigens presented in the context of MHC molecules. With the advent of hybridoma and T cell cloning technologies, they have finally succeeded.

T cell receptor is a heterodimer composed of α and β subunits, each of relative molecular weight of approximately 45,000. Another gene, termed γ has also been cloned and characterized although the protein product of this γ gene has still not been isolated. The function of the γ gene is unknown although it has been suggested (Heilig et al. 1985) that it may have a role in induction of self tolerance during ontogeny.

The interesting fact which has emerged is that the primary structure of the α, β, and γ T cell receptor genes are similar to that of the immunoglobulin genes and can also undergo somatic rearrangement. The T cell receptor genes are assembled from separate segment, the constant region C, the variable region V, the diversity region D and the joining region J. There are individual differences between the three T cell receptor genes and the immunoglobulin genes (Hood et al. 1985) which may account for individual specificities (T-helper vs. CTL).

Now that the T cell antigen receptor has been identified, isolated, and the genes encoding the molecules cloned, it appeared likely that finally a picture would emerge which would explain in definitive terms the mechanisms of T cell recognition of antigen and MHC. But there is still no evidence in the literature to indicate that the T cell receptor can bind to either the antigen or the MHC. Schwartz (1985) has proposed two models to explain the interaction...
between antigen and Ia molecule during T cell activation. The first model postulates that the antigen and the Ia molecule interact first, and then the T-cell receptor interaction takes place. The second model visualizes that the reverse may be true. The bulk of the evidence from Schwartz’s laboratory favors the first hypothesis.

MHC GENES AND GENETIC CONTROL OF DISEASE STATES

MHC genes exert a major influence on whether an individual is resistant or susceptible to particular disease states. MHC class II genes have been implicated in disease states such as thyroiditis, allergic encephalomyelitis, myasthenia gravis, and collagen induced arthritis. The principal cause of the pathology of the above mentioned disease states is the abnormal reactivity of the immune system to self antigens.

Normal individuals do not react to self antigens because during ontogeny T cells reactive to self antigens have been deleted. Autoimmunity may, however, be elicited if this tolerance is broken due to viral or bacterial infections or due to other means of modification of self antigens. Autoimmunity may also be evoked if there is a disruption in suppressor T cell function. Suppressor T cells function as regulating agents and keep in check clones of autoreactive B or T cells.

The association between a gene, its products and susceptibility to disease is of paramount importance in our understanding the genetic basis of disease. It can, for example, predict the probable risk of a certain disease in genetically defined group of individuals. For instance, in the induction of experimental autoimmune myasthenia gravis (Christadoss et al. 1981) the H-2^B strains of mice are highly susceptible whereas mice of the H-2^K haplotypes are the least susceptible. In the induction of collagen-induced arthritis (Wooley et al. 1981), only mice of the H-2^K haplotype were most susceptible to disease. In both the above disease states, it was postulated that the Ia antigen on the macrophage could effectively present the respective antigens (acetylcholine and chick type II collagen) to initiate an anti-self response. Although the mechanism(s) for the association of MHC antigens to disease states are unclear, evidence for their involvement is overwhelming (Long et al. 1984).

Autoimmune thyroiditis, insulin dependent diabetes mellitus (IDDM), myasthenia gravis, Addison's disease and rheumatoid arthritis (RA) are all characterized by autoimmunity (autoantibodies and perhaps cytotoxic T cells) and an association with HLA-DR3 and/or DR4 (IDDM and RA) (Svejgaard et al. 1983). These diseases also have a tendency to cluster in the same patient and in the families suggesting that susceptibility to autoimmunity lies in HLA-DR3, DR4 or perhaps other closely linked DQ and DP MHC class II genes. But how MHC class II genes lead to autoimmunity is an enigma. It is logical to think that certain environmental events in HLA-DR3, DR4 carrying individuals lead to the presentation of 'self' antigens to T helper lymphocytes and autoimmunity against these antigens. B cells which express Ia on their surface can also bind antigen through their specific surface immunoglobulin and present it to MHC restricted cloned T cells (Lanzavechia 1985). In fact, MHC class II molecules alone inserted on synthetic lipid vesicles with antigen bound covalently to the lipid molecules can also stimulate cloned helper T cells and T cell hybridomas in antigen-specific MHC-restricted manner in absence of APC's (Walden et al. 1985). Therefore it is not inconceivable that an abnormal tissue distribution
lead to initiation of immune response against the antigens of particular tissue where these antigens are expressed because normally class cells, Langerhans cells - B lymphocytes activated T cells and vascular endothelial cells (Unanue 1984).

Botazzo and colleagues have recently demonstrated the presence of DR antigens on thyroid cells from patients with Grave’s disease and on beta islet cells in pancreas from a patient with insulin dependent diabetes mellitus (Hanafusa et al. 1983, Botazzo et al. 1985). They have hypothesized that the ‘aberrant’ expression of DR in these tissues leads to presentation of autologous antigens to T helper lymphocytes and thus triggers autoimmunity (Botazzo et al. 1983). Abnormal suppressor cell function found in many autoimmune diseases is explained as a secondary phenomenon in this hypothesis. They have since provided evidence to support their hypothesis. They cultured thyrocytes expressing HLA-DR from a patient with Grave’s disease and demonstrated that these cells could present peptide p20 of influenza haemagglutinin to antigen specific cloned T cells (Londei et al. 1984). In another study they also showed that cloned helper T lymphocytes from thyroid gland of a patient with Grave’s disease recognized the autologous thyroid cells but not DR mismatched thyroid cells (Londei et al. 1985). They have postulated that viral infections and other environmental factors lead to local release of interferon which induces the expression of HLA-DR in these tissue cells. HLA-DR3 may be predisposing to viral infections or may be more easily inducible by the interferon and thus leading to autoimmunity in this way.

However, until mRNA of DR genes is demonstrated in these non-lymphoid cells, one cannot be certain that HLA-DR antigens are not passively absorbed onto surface of these cells. Even then it is possible that the expression of class II antigens on these cells is due to effector T cell response against these cells, i.e. interferon released by activated T cells. And then, why should local release of interferon lead to selective expression of Ia only on beta cells and not other islet cells or exocrine pancreatic cells?

Notwithstanding these objections, it is an elegant hypothesis to explain how certain MHC genes produce autoimmunity. It is possible that the ’defect’ may be in regulatory sequences or promoters of HLA-DR3, DR4 (or closely linked DQ and DP) genes, which may be easily derepressible by interferon in non-lymphoid tissues leading to the ‘aberrant’ expression of these genes. The same ‘defective’ promoter may be with majority of DR3 and DR4 genes but also with some other DR genes and that may be the reason why all patients with autoimmune diseases do not carry HLA-DR3, DR4.

Most of the current knowledge we have today on how the immune response takes place in an individual is based on in vitro studies in the laboratory. No doubt these have been elegant studies using state of the arts technology in cellular immunology utilizing monoclonal antibodies, cloned T cell lines, hybridomas, cell sorter and finally recombinant DNA techniques. Yet, eventually one has to go back to the whole animal to decipher what happens when a foreign antigen enters ’in vivo’. A whole animal is still a ’black box’. One way to understand the role of the immune system is to look at actual disease situations. Our laboratory has been involved for many years in studying how the immune network functions in several diseases. We will briefly describe our studies to date.
1) Experimental Autoimmune Thyroiditis

There exist several useful animal models of spontaneous and induced autoimmune thyroiditis. The spontaneous disorder, accompanied by autoantibody production, occurs in dogs, monkeys, rats and chickens [Weigle 1980]. The disease was first induced in rabbits injected with an adjuvant and homologous thyroid extract or thyroglobulin (Tg) [Witebsky and Rose 1956]. Subsequently, the disorder has been produced in rabbits, dogs, guinea pigs, goats, rats, chickens, monkeys and mice [Weigle 1980]. We will limit our consideration to the murine model, with which our lab has had experience. We will discuss the murine disease in relation to its human prototype, Hashimoto’s thyroiditis.

Experimental autoimmune thyroiditis is most commonly induced in mice by injections, into the hind footpads, of aqueous heterologous mouse Tg incorporated into complete Freund’s adjuvant (Rose et al. 1971). Generally, two injections of 0.1 ml of complete Freund’s adjuvant containing 20-80 μg of Tg are administered one week apart. The animals are sacrificed several weeks after the first antigenic challenge, and the entire thyroid is sectioned for histologic examination. It should be noted that Tg is a normal secretory component of the thyroid gland, its secretion being under pituitary regulation. Measurable Tg is present in normal serum, and thus it cannot be considered a sequestered antigen [Van Herle et al. 1979, Van Herle et al. 1979].

Histologically, the experimental thyroid lesion is characterized by an early granulocytic infiltration followed by a marked interstitial lymphocytic infiltration. Occasional lymphoid accumulations resembling germinal centers are seen. Sparse macrophages and plasma cells are found in the perifollicular tissue. Occasionally, there is frank destruction of the gland with fibrosis of the follicular epithelial cells [Weigle 1980]. Immune complexes containing complement-fixing antibodies of the IgG class are found at the basal area of the follicular cells. Thyroglobulin is one of the antigens found in these immune complexes [Clagett et al. 1974].

The human disease bearing the strongest resemblance to experimental murine thyroiditis is Hashimoto’s thyroiditis. Characteristically, this disorder results in a slowly progressive loss of thyroid hormonogenic capacity [DeGroot et al. 1984]. Histologically, the thyroid is markedly infiltrated with lymphocytes and other mononuclear cells. There are lymphocytic follicles apparent in the interstitium of the gland. The total number of thyroid follicles is decreased, and those present are practically devoid of colloid. Fibrous thickening of the interlobular septa is common. Hurthle cells, oxyphilic cells with prominent nucleoli, are characteristic of Hashimoto’s thyroiditis [Knecht et al. 1981]. Hurthle cells have not been described in experimental murine thyroiditis. Immune complexes, presumably formed in situ have been localized to the basal follicular basement membrane in the human disease by fluorescence microscopy. As in the murine model, these immune complexes fix complement and are thereby effectors of tissue damage [Kalderon and Bogaars 1977].

Mice immunized with purified heterologous mouse Tg produce circulating antibodies to both heterologous and autologous Tg [Nakamura and Weigle 1968]. A good correlation does not exist between murine Tg antibody titer and the severity of the thyroid lesion [Tomazic and Rose 1977]. Tg antibodies, as well as microsomal antibodies, are practically ubiquitous in the serum of patients.
with Hashimoto's thyroiditis (Doniach et al. 1978). These human antibodies directed against thyroglobulin are mainly of the IgG class, with rare IgA and IgM antibodies being present (Delespese et al. 1976). As in the murine model, not all individuals with Tg antibodies have thyroiditis and the antibody titer does not predict the severity of the disease. Other thyroid specific antibodies have been found in Hashimoto's thyroiditis, including some which block thyroid stimulating hormone's binding to its receptor (Endo et al. 1978). This is a possible mechanism, besides glandular destruction, for the hypothyroidism. Additionally, growth stimulating immunoglobulins, which may contribute to the glandular enlargement, have been identified (Drexhage et al. 1980). None of these latter antibodies have been described in experimental thyroiditis.

Murine thyroiditis was the first autoimmune disease in which a strong major histocompatibility complex (MHC) influence could be demonstrated. Tg-immunized mice only having the H-2 region in common show a similar degree of thyroid involvement. Specifically, mice bearing the H-2^k allele (CBA/J strain) are good responders; those with the H-2^d or H-2^d (BALB/c strain) are poor responders (Vladutiu and Rose 1971). Within the murine MHC, located on chromosome 17, the major control gene for experimental autoimmune thyroiditis (Ir-Tg) has been localized to the I-A subregion. This gene determines the initiation of the response (Beisel et al. 1982). D-region and possibly K-region gene products interact with the I-region products to modify the production of the thyroid lesion. It appears likely that the D-end regulates the suppressor T cells which modulate the cytotoxic effector cells of thyroid damage. It is also possible that D-end genes play a role in the synthesis of complement, another system involved in the thyroid lesion (Kong et al. 1979).

The demonstration that MHC genes control the susceptibility to and severity of experimental thyroiditis led to the search for an association of goitrous Hashimoto's thyroiditis with human leukocyte antigens (HLA). In a large Caucasian population, there were found no specific deviations in HLA-A, B or C frequencies in individuals with this condition. However, a significant increase in HLA-DR5 was seen (RR=3.1). In contrast, atrophic thyroiditis was found to be associated with DR3 (RR=5.7) and Graves' disease with HLA-B8 (RR=2.65) (Farid and Bear 1981). This HLA-DR association in Hashimoto's thyroiditis is homologous to the H-2 I-A subregion localization of the major control gene for murine thyroiditis.

The theory of clonal balance, the equilibrium between autoreactive T helper and T suppressor cells necessary to maintain self tolerance, is supported by the murine model of thyroiditis (Weetman and McGregor 1984). In good responder mice, the injection of two doses of Tg prior to the standard immunizing regimen induces tolerance to Tg. Tolerance can also be effected by raising endogenous Tg by thyroid stimulating hormone or thyrotropin-releasing hormone injections prior to immunization. This tolerogenic regimen is thought to activate Tg-reactive T suppressor cells since spleen cells from tolerant mice can transfer tolerance to normal syngeneic recipients (Kong et al. 1982). Suppressor T cells are especially vulnerable to cyclophosphamide. It has been demonstrated that poor responder strains treated with cyclophosphamide prior to antigenic challenge can be converted to high responders (Vladutiu 1982).

There is evidence that clonal balance is disrupted in patients with Hashimoto's thyroiditis. An antigen (Tg) specific defect in T suppressor cell function without a general T suppressor abnormality has been demonstrated (Okita
et al. 1981). In addition, some investigators have reported decreased percentages of OKT8 or Leu 2 cells (generally considered suppressor/cytotoxic cells) in the circulation of patients with the disorder (Raeman et al. 1981, Iwatani et al. 1983). An examination of thyroid-infiltrating T lymphocyte subsets in Hashimoto’s thyroiditis demonstrated proportionally fewer OKT8 cells in the goiters than were found in the peripheral blood (Jansson et al. 1983).

Although Tg antibodies are a necessary component of the murine autoimmune response to Tg, they are not sufficient to produce the thyroid lesion; poor responder mice with minimal thyroid lesions, may have high titer Tg antibodies (DeCarvalho and Roitt 1982). The ability to induce thyroiditis in good-responder mice by passive transfer of immune serum suggests the importance of immune complexes in the development of the thyroid lesion. The fixation of complement to these complexes appears to mediate thyroid damage (Tomazic and Rose 1975). The pattern of localization of complement-fixing immune complexes to the basal follicular basement membrane in experimental thyroiditis (Clagett et al. 1974) is quite similar to that seen in Hashimoto’s thyroiditis (DeGroot et al. 1984).

Another effector of thyroid damage in the murine model appears to be direct T cell cytotoxicity. Lymph node cells from Tg-immunized mice are cytotoxic to syngeneic thyroid cells in vitro when activated with murine Tg or thyroid cells (Kong et al. 1986). A similar cytotoxicity of peripheral leukocytes from patients with Hashimoto’s disease against human thyroid cells has not been demonstrated (Chow et al. 1983). As discussed previously, the suppression of cytotoxic effector cells and the synthesis of complement, both effects of thyroid damage, may be under D-end gene control in the mouse. In the human, no similar MHC class I (HLA-A,B, or C) association has been identified (Farid and Bear 1981).

2) Experimental Autoimmune Myasthenia Gravis (EAMG)

Myasthenia gravis is a disease of the neuromuscular junctions in the body, in which a reduction in the number of acetylcholine receptors in the postsynaptic membranes by autoimmune mechanisms leads clinically to progressive weakness of the skeletal muscles on exercise (Fambrough et al. 1973, Lennon and Lambert 1980). The disease has been induced experimentally in rats (Lindstrom et al. 1976) and mice (Fuchs et al. 1976) by intradermal injection (with adjuvants) of acetylcholine receptors (AChR) extracted from the electric organ of eels or Torpedo rays, or from syngeneic muscles. EAMG resembles the human disease clinically, electrophysiologically and immunologically, with a high correlation between in vitro lymphocyte responses to Torpedo AChR, serum autoantibodies to AChR, endogenous muscle complexed AChR antibodies, and the susceptibility to the disease. All of the above immune responses are independent of the dose of the administered antigen. It was soon clear that the immune responses and susceptibility to the disease were controlled by a Mendelian dominant gene mapping to the MHC. Christadoss et al. (1979,1981) demonstrated that in H-2 congenic mice with B10 or B6 backgrounds, H-2^d haplotypes were high responders and susceptible to the disease, whereas those with H-2k,P,q,s haplotypes were low responders and disease resistant. The immune response and susceptibility genes were further mapped to the I-A subregion of the H-2 complex by backcross studies and using recombinant strains. This was confirmed additionally by the fact that a point mutation in the I-A subregion (Ag locus) in the B6 strain (B6.C-H-2^bm12) converted a state of high responsiveness and
susceptibility to low responsiveness and resistance. Moreover, lymphocyte responses in vitro in high responders, were eliminated with specific anti-I-A alloantisera. A non-H-2 gene too seemed to govern immune responses in EAMG when it was shown that inbred AKR (H-2K) strains produced high levels of autoantibodies to AChR and seemed to be susceptible to the disease, although H-2K strains in general were low responders and disease resistant. However, the AKR mice did not show any in vitro lymphocyte proliferation to AChR. This paradoxical situation could be explained by an immunomodulating effect of circulating murine leukemia viruses, seen spontaneously in these strains of mice. A similar explanation could be invoked for anomalous high responder status of SJL (H-2S) strains of mice, which are known to develop lymphomas and thymomas spontaneously, possibly induced by viruses.

3) Trichinellosis

Allelic polymorphism of MHC genes also influences the immunity to parasite infestations in animals. Studies on Trichinella spiralis infestation of mice in our laboratory have shown that H-2 genes affect the degree of resistance to this parasite. The disease in humans is acquired through the ingestion of improperly cooked pork. The adult worms reside in the intestinal mucosa from where they release larva into the bloodstream which finally reach and get encysted in tissues like skeletal muscle, heart, and brain. Resistance to Trichinella spiralis is manifested by the active expulsion of the developing or the adult worm from the intestine and requires antibodies, T lymphocytes and eosinophils (Love et al. 1976, Kazura and Aikawa 1980). Trichinella spiralis infection in immunocompromised patients can be fatal. Using H-2 congenic B10 mice we found that mice with q haplotype were more resistant to infection than those with f and k haplotype (Wassom, et al. 1979). In both primary and challenge infestations with T. spiralis, B10.Q (H-2^q) mice expelled the worms more quickly than B10.M (H-2^f) which in turn did it faster than B10.BR (H-2^k). H-2 genes influence not only the rate of expulsion of worms but also their fertility because worms in B10.BR (H-2^k) produced more larvae than worms in B10.M (H-2^f) and B10.Q (H-2^q) mice (Wassom et al. 1983). Studies with recombinant mice mapped the resistance to Trichinella spiralis infection in mice to two MHC genes: A^r and a second gene in between S and D genes (Wassom et al. 1980, Wassom et al. 1983). The exact role of H-2 genes in immunity to this parasite is being further defined by 'in vitro' studies with Trichinella spiralis specific T cell clones from resistant and susceptible strains and monoclonal antibodies to H-2 antigens. Trichinella spiralis has several antigens and perhaps different H-2 antigens induce the immune response to these different antigens, which in turn may determine the degree of resistance to the parasite. Genes outside the MHC also influence the susceptibility to Trichinella infection in mice, because H-2 identical strains of differing backgrounds differ in their ability to expel the worms. Although no human parasite infection has been linked to HLA, what makes this model in mice interesting to study is the interaction between MHC and non-MHC genes in immunity against this parasite. Whether some of these non-MHC genes are involved in eosinophilic function is a matter of conjecture at present.

4) Collagen Induced Arthritis In Mice

Collagen induced arthritis (CIA) is an experimental model of chronic inflammatory arthritis induced by intradermal injection of type II collagen in Freund’s adjuvant in rats (Trentham et al. 1977) mice (Courtenay et al. 1980),
and primates (Gonnermann, et al. 1984). It is characterized by the development of cellular and humoral immune responses to type II collagen, which is the major component of hyaline cartilage in the joints. The disease bears numerous clinical, pathological, immunological and genetic similarities with rheumatoid arthritis in humans (Trentham 1982). Susceptibility to CIA is seen to be associated with the major histocompatibility complex genes in both rats (Griffiths et al. 1981, Griffiths 1981) and mice (Wooley et al. 1981, Wooley et al. 1983) and diseased animals have high levels of anti-type II collagen antibodies. Using inbred, hybrid, congenic and recombinant strains, the susceptibility to chick type II CIA in mice has been mapped to the I region of H-2^q haplotype (Wooley et al. 1981), and specifically to the I-A subregion (Huse et al. 1984) in congenic strains with B10 background. It has also been found that H-2^q haplotype mice are resistant to porcine type II (PII) CIA, in contrast to H-2^f haplotype mice which are resistant to chick type II (CII) but susceptible to porcine type II CIA when both strains of mice have a B10 background. These results suggest the possibility of at least two arthritogenic epitopes on type II collagen molecule (Wooley et al. 1985). In F1 mice of (susceptible x resistant) strains congenic to B10 background, the susceptibility is inherited as a codominant trait. Inbred strains also indicate non-MHC involvement, since DBA/1J (H-2^b) are susceptible to both PII and CII, while SWR/J (H-2^q) and RIII (H-2^f) are resistant to the disease by both collagens. Transfer of the disease into naive mice has been possible using sera from arthritogenic mice (Stuart and Dixon 1983, Wooley et al. 1984). The disease has also been transferred into naive mice with affinity purified anti-type II collagen IgG from a patient with seronegative rheumatoid arthritis (RA) (Wooley et al 1984) demonstrating that anti-collagen type II antibodies, described in 30-70% patients of RA, may play an important, if not primary, role in the etiopathogenesis of at least a subset of RA patients. Modification of CIA in mice has been observed by pretreatment of animals with i.v. native type II collagen, polyclonal and monoclonal type II collagen antibodies (Wooley et al. 1984) and polyclonal and monoclonal anti-Ia antisera (Wooley et al. 1985).

**COLLAGEN-INDUCED ARTHRITIS**

![Collagen-Induced Arthritis Diagram](image-url)
Figure 5 shows our current hypothesis on how arthritis is induced in these mice with type II collagen. Obviously, the collagen molecule must have several antigenic sites or epitopes. Most mice will make antibodies against one or more of these epitopes, but only when immune response is generated against an epitope which causes reactivity against the exposed epitope on the animal’s own cartilage will the disease be induced. In mice, the H-2^q haplotype mice generate an antibody against an arthritogenic epitope. The IA^q presents this epitope to the T helper cell whose antigen receptor sees the antigen in the context of IA. In some cases, the macrophage or antigen presenting cell could secrete IL-1 (interleukin-1) which may directly attack the joint tissues. The stimulated T helper cell is known to secrete IL-2 to turn on other T cells and B cells. This usually happens through the IL-2 receptor. These stimulated T cells then could take different pathways. They could turn on the B cell to produce antibodies which would attack joint tissue. They could stimulate the effector mechanism for cellular immunity. In some cases they could turn on the suppressor T cells which would neutralize the immune response. The antibodies which are generated against non-arthritogenic epitopes would have no role in causing the disease. Immunotherapy in the collagen arthritis model could be performed in several different stages. Injection of anti-IA could prevent antigen presentation. Since most T helper cells express the marker L3T4, in vivo therapy with anti-L3T4 could deplete these T cell population causing a reduction in the immunity. Antibodies could be injected against the IL-2 receptor and would prevent the cells from blast transformation. Injection of anti-idiotype antibodies or anti-clonotype antibodies could prevent the T-cell receptor from recognizing the antigen, down regulate antibodies of the pathogenic idiotype, or stimulate T suppressor cells.

5) Theiler’s Murine Encephalomyelitis

Theiler’s murine encephalomyelitis (TME) is an important model for the study of viral persistence in the nervous system and immune mediated demyelination (Theiler 1937, Lipton 1975). This naturally occurring disease in mice has been considered to be an excellent model of multiple sclerosis because demyelination in the human disease may be the result of an immune mediated response triggered by a persistent virus. The destruction of white matter is associated with inflammatory cells which play an active role in myelin breakdown so the lesions are fewer and less severe in TME virus infected mice treated with immunosuppressive agents or with monoclonal antibodies directed at immune response gene products (Lipton and Dal Canto 1976, Roos et al. 1982, Rodriguez et al. 1985). Early studies demonstrated a wide range of genetic susceptibilities to TMEV induced demyelination in mice from different strains (Lipton and Dal Canto 1979, Lipton and Melvold 1984). We have shown, using morphologic criteria, that mice with similar genetic backgrounds but different H-2 haplotypes have different susceptibilities to demyelination. Using mouse strains with congenic recombinant haplotypes, we have demonstrated that the D region of the H-2 complex is primarily responsible for determining susceptibility or resistance to TMEV infection. In addition, a mutation at the H-2D gene alters the susceptibility to virus induced demyelination. There is a correlation between the ability of TMEV to persist in the central nervous system and the TMEV specific humoral response with genetic susceptibility.
Our current hypothesis on the Theiler virus induced demyelination in susceptible strains of mice. When virus is injected into the brain of the mice, it is processed by an antigen presenting cell and presented in the context of the D gene. In a resistant strain, cytotoxic T lymphocytes are generated which kill the neural cells infected with the virus and the animals do not get the disease. In the susceptible strain, either the antigen is not presented by the particular D gene or the T cell fails to recognize the presented antigen. Thus, minimal or no cytotoxic cells are generated. The virus continues to infect first the astrocytes and then the oligodendrocytes. After chronic viral infection of the oligodendrocyte, a couple of things happen. Remyelination is affected and an altered myelin is produced. The virus, either through interferon induction or by gene fusion, causes the expression of class II molecules on the oligodendrocytes. These class II molecules now present the altered myelin to the T helper cell which recognizes it as 'non-self' and generates the production of autoreactive T cells as well as autoantibodies which in turn cause the destruction of the myelin.

THERAPY OF AUTOIMMUNE DISEASE WITH ANTI-Ia ANTIBODIES

Treatment with antibodies directed against Ia antigens has been shown in several animal models to modulate the disease by delaying onset and decreasing the severity of even established disease. Since several of the human autoimmune diseases are associated with increased incidence of HLA-DR antigens it would seem desirable that this approach be used as therapy of human disease.

Steinman et al. (1981,1983) reported their experience using anti-Ia antibodies in the experimental allergic encephalitis (EAE) model of demyelinating disease of the nervous system. This disease is T cell mediated and is
controlled in part by genes of the major histocompatibility complex. In their studies they used several different types of polyclonal and monoclonal antibodies against I region gene products. The animals were treated either at the time of immunization or a few days later. The results showed that animals given antisera directed against the susceptible haplotype namely IS had less weight loss, less evidence of clinical EAE, milder disease and less severe histological changes. The time of onset of the disease was not altered except in those animals given antisera after immunization.

Waldor et al (1983) reported similar results using monoclonal anti-Ia antibodies in the myasthenia gravis model. In these animals they showed that both humoral and cellular immune responses were suppressed. Carrying these studies further Adelman et al. (1983) looked at the effect of anti-Ia therapy on the renal disease in the NZB/W F1 model of systemic lupus erythematosus. Renal disease is tightly linked to the H-2\(^Z\) haplotype. They showed that antibodies directed against Ia\(^Z\) haplotype induced longlasting remission in female mice with moderate renal disease. Anti-Ia therapy directed against the H-2\(^D\) haplotype did not have the same effect.

In 1985 Wooley et al. (1985) reported the experience using multiple anti-Ia antibodies native in the type II collagen induced arthritis model of rheumatoid arthritis. We have previously shown that susceptibility to this antigen is controlled by H-2\(^Q\) genes at the I-A region (Huse et al. 1984). Several different monoclonal and polyclonal anti-Ia antisera which had specificity against different Ia antigens but did have cross-reactivity with H-2\(^Q\) were used. Animals were treated the day before, the day of, and the day after immunization. It was observed that several monoclonal antibodies which had significant cross-reactivity with Ia\(^Q\) in a cytotoxicity assay were most active in inducing suppression of arthritis in B10.Q mice. Those antisera which were poorly reactive had less suppressive effect. Interestingly, few antisera enhanced the arthritis as evidenced by higher titers of anti-CII antibodies, earlier onset and a higher incidence. In general, there was no effect on delayed type hypersensitivity. Antibody titers at 14 days were suppressed, but normalized at 28 days.

Thus, several studies have shown that antibodies directed against Ia antigens can suppress both the humoral as well as cellular immune response dependent on that Ia antigen, although the results can be variable depending upon the type of antibody used and the type of animal system studied. In some circumstances the suppression is haplotype specific leaving immune response of these animals intact to other antigens. The fact that certain antibodies were able to enhance the disease should caution us about choosing the antibody for treatment. None of the animals suffered any unusual side-effects in terms of weight loss, loss of hair, malignancy or death. Any changes observed in these animals could be explained on the basis of the underlying disease process. Finally, the suppression of the disease has been observed both in early as well as in established disease.

FUTURE STUDIES

If the aberrant tissue expression of HLA-DR3, DR4 leads to autoimmunity because of the defective gene promoter which is easily induced by interferon, then this hypothesis can be tested by producing inappropriate tissue expression of Ia genes in transgenic animals. These animals with foreign DNA integrated
into their genome are produced by microinjecting foreign genes into the fertilized ova and then developing the ova to maturity in foster mothers. Many studies in transgenic animals, mostly mice, have shown that tissue expression of any gene is determined by the regulatory or promoter sequence attached to the gene (Palmiter and Brinster 1985, Brinster et al. 1985, Gordon and Ruddle 1985). Therefore, abnormal tissue expression of Ia genes in thyrocytes or pancreatic beta cells can be produced by ligating Ia genes to thyroglobulin or insulin gene promoter and microinjecting these gene constructs into the fertilized ova: if transgenic animals thus produced develop autoimmunity, the hypothesis will be proved.

Transgenic animals are a powerful tool to study not only gene regulation and expression but also to define the role of any gene in producing a particular disease. For example, by producing mice transgenic for HLA-B27 it should be possible to find out if B27 gene alone is sufficient to produce ankylosing spondylitis or other HLA-B27 associated arthritides.

Another possible mode of therapy in MHC linked diseases in the future is going to be gene therapy. By introducing disease resistant MHC genes into disease susceptible (MHC linked) strains, it may be possible to prevent or cure the disease. The first step in this direction will be to microinject disease resistant MHC genes into fertilized ova from mice with susceptible strains and then testing the transgenic mice thus produced for the occurrence of disease. If such experiments with germ line gene therapy are successful, then specific tissue tropic vectors could be designed to introduce genes into the somatic cells of mature animals. It may also be possible to 'switch off' the disease producing genes by introducing 'anti-sense' genes, i.e. gene promoter attached to the anti-sense strand of the gene! Such inverted genes should transcribe anti-sense mRNA because the gene promoter will be on anti-sense strand and anti-sense mRNA will block translation of gene product by hybridizing to sense mRNA. This type of 'anti-gene' therapy may be especially applicable to autoimmune disease if the autoimmunity is because of inappropriate tissue expression of MHC genes.

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