Genetic Predisposition of Autoimmune Disease and B-Cell Chronic Lymphocytic Leukemia (B-CLL)

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— Autoimmune disease is a polygenic disease in which various genetic factors play crucial roles. The familial clustering and the association of HLA haplotypes have been well-recognized. There is a close association between chronic lymphocytic leukemia (CLL) and autoimmune disease. Like autoimmune diseases, CLL is the type of leukemia most often occurring among close relatives. The patients with CLL frequently share common HLA haplotypes with relatives with autoimmune disease. As the majority of CLL is of CD5⁺ B-cell type, and as CD5⁺ B cells are suggested to be involved in autoimmunity, certain regulatory abnormalities in the proliferation and differentiation of CD5 B cells may be involved in both B-CLL and autoimmune disease. I discuss here the possibility that different, but related, MHC haplotypes would predispose either to autoimmune disease or to B-CLL, based on our findings obtained from MHC (H-2)-congenic New Zealand mouse strains. ——— autoimmunity; B-CLL; CD5 B cell; New Zealand mice; SLE

It has long been suggested that the autoimmune disease and lymphoid malignancy are related events. However, the mechanisms underlying these events have been a matter of conjecture. Autoimmune disease is a polygene system disease in which various genetic factors play crucial roles. The familial clustering of disease has been well-recognized. The association of HLA haplotypes suggested that genes in the major histocompatibility complex (MHC) act as one major predisposing genetic element.

Among lymphomas/leukemias, the chronic lymphocytic leukemia (CLL) has been reported to be the type of leukemia most often occurring among close relatives (Conley et al. 1980). There is also an association between CLL and HLA haplotypes, the finding in keeping with that of autoimmune disease. The most intriguing finding is that one or more autoimmune diseases develop either in patients with CLL or in their family members. Together with the findings that the majority of CLL is of CD5 B (B1) cell type and that CD5 B cells do produce

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autoantibodies (Hayakawa et al. 1983), it is feasible that certain regulatory abnormalities in the proliferation and differentiation of CD5 B cells may be involved in both autoimmune disease and B-CLL. This is indeed the case in families of New Zealand strains of mice, in that either autoimmune disease or CD5⁺ B-CLL develops, depending on the difference in genetic backgrounds (Shirai et al. 1991a, 1992).

Autoimmune disease in New Zealand mouse strains are polygenic

The inbred New Zealand mouse strains were established on the basis of coat-color selection from the original outbred, agouti-colored mice derived from Imperial Cancer Research Fund, Mill Hill, London (Howie and Helyer 1968; Shirai et al. 1991b). The black-colored NZB strain, developed in 1959 by Marianne Bielschowsky in New Zealand, is the first available, spontaneous animal model of autoimmune disease, and develops autoimmune hemolytic anemia. The white-colored NZW strain does not develop autoimmune disease. However, when these NZW and NZB strains were cross-bred, the F₁ hybrid (NZB/W F₁) mice develop the disease resembling human systemic lupus erythematosus (SLE) without apparent autoimmune hemolytic features.

These findings are of particular importance with respect to the etiopathogenesis of autoimmune disease. What we have first learned from these mice is that the autoimmune diseases are a genetically-determined disease, because these mice are all inbred and because such autoimmune diseases always develop through generations and generations. Second, the autoimmune diseases are polygenic, because SLE occurs only in NZB/W F₁ mice and because autoimmune hemolytic anemia characteristic of NZB strain does not develop in the F₁ hybrid. Third, normal individuals may carry genes which modify the phenotypes of autoimmune disease, because the features of autoimmune disease between NZB and NZB/W F₁ mice differ.

Our genetic progeny studies showed that the autoimmune diseases in these mice are indeed polygenic. The genetic control of autoimmune hemolytic anemia can be interpreted by two loci on chromosome 4 (Ozaki et al. 1983). One of them is a dominant predisposing gene and is unique in NZB strain. The other is a dominant suppression gene and preexist in most strains of mice including the NZW, but not in the NZB strain. Thus, in the NZB/W F₁ mice autoimmune hemolytic anemia is suppressed by the contribution of the second gene from NZW strain.

The mode of genetic inheritance of SLE in NZB/W F₁ mice is different from that of autoimmune hemolytic anemia and at least 5 genes are likely involved (Shirai et al. 1987 and unpublished observation). Progeny studies revealed that the two of 5 genes are tightly linked to the H-2 complex on chromosome 17, one with H-2^d of NZB and one with H-2^z of NZW. Linkages of two other genes were also determined and one was linked to the T cell receptor (TCR)- β chain gene complex on chromosome 6 of NZW (Yanagi et al. 1986; Hirose et al. 1991) and one

to the TCR- α chain gene complex on chromosome 14 of NZW strain. Another NZB gene was found to be located on the telomeric portion of chromosome 4 (Hirose et al. 1994).

To confirm the linkage to and examine the role of the H-2 complex in the development of SLE, we developed H-2-congenic NZB and NZW strains, in which H-2^d haplotype of NZB was introduced to NZW, and H-2^z haplotype of NZW to NZB strain, by selective backcrossing for 12 generations. By mating these H-2-congenic and original NZB and NZW strains, we produced 4 types of NZB/W F₁ mice; heterozygous (NZB.H-2^d × NZW.H-2^z)F₁, (NZB.H-2^z × NZW.H-2^d)F₁, homozygous (NZB.H-2^d × NZW.H-2^d)F₁ and (NZB.H-2^z × NZW.H-2^z)F₁. What happened was that the development of fatal immune-complex type glomerulonephritis (lupus nephritis), as determined by proteinuria and IgG-class anti-DNA antibodies (both characteristic of SLE), occurred only in H-2^{d/z} or H-2^{z/d} heterozygotes (Hirose et al. 1986, 1990). Thus it is clear that the heterozygosity of H-2^d and H-2^z is required for the development of SLE in NZB/W F₁ mice. Nonetheless, it was also obvious that other genetic predisposing elements also contribute to the generation of disease, because SLE did not occur in either NZB or NZW mice with H-2^{d/z} heterozygosity (Table 1). All these findings taken collectively demonstrate that similar to human SLE, the murine SLE in New Zealand mice is a polygene disease and shows the familial clustering, in which a particular MHC haplotype acts as one major predisposing genetic element.

B-CLL in New Zealand mouse strains

During the studies on H-2-congenic strains of New Zealand mice, we found that homozygous H-2^{z/z} New Zealand mice including NZB, NZW and NZB/W F₁ all show extremely high frequencies of CD5 B cells in the spleen (Okada et al. 1991). With increasing age of these mice, such high frequencies of CD5 B cells also appeared in the blood and blood smears revealed to have characteristics of B-CLL. In fact, the infiltration of these leukemic cells was observed in a variety of organs including lymph nodes, spleen, lung, liver, salivary glands and so on. The neoplastic nature of these cells was given support by the transplantability of these cells into young syngeneic mice. Furthermore, the message expression of proto-oncogene *bcl-2* was markedly increased in accordance with the observation in human B-CLL (Okamoto et al. 1993).

CD5 B Cells in autoimmune disease and B-CLL

What is the mechanism underlying the clustering of autoimmune disease and B-CLL in different members of the New Zealand mouse family? Autoimmune disease like SLE is a disorder of abnormal differentiation of autoreactive B cells. By contrast, the majority of B-CLL is a disease of aberrant proliferation of CD5 B cells, mostly potentially capable of producing autoantibodies (Hayakawa et al. 1983). Therefore, given that the pathogenic autoantibodies in SLE such as

TABLE 1. *A model of contribution of NZB and NZW genes to the production of anti-DNA antibodies in NZB/W F₁ mice*

Mice	H-2	Putative genes					Anti-DNA antibodies		Proteinuria
		NZB		NZW			IgG	IgM	
		Ads-1 (H-2 ^a)	Ads-2 (?)	Ads-3 (H-2 ^z)	Ads-4 (TcR β)	Ads-5 (TcR α)			
NZB	d/d	+	+	-	-	-	-	#	+
NZB. H-2 ^z	z/z	-	+	+	-	-	-	#	±
NZB × NZB. H-2 ^z F ₁	d/z	+	+	+	-	-	-	#	+
NZW	z/z	-	-	+	+	+	-	-	-
NZW. H-2 ^d	d/d	+	-	-	+	+	-	-	-
NZW. H-2 ^d × NZW F ₁	d/z	+	-	+	+	+	-	-	-
NZB × NZW F ₁	d/z	+	+	+	+	+	#	+	#
NZB × NZW. H-2 ^d F ₁	d/d	+	+	-	+	+	-	+	+
NZB. H-2 ^z × NZW F ₁	z/z	-	+	+	+	+	-	+	-
NZB. H-2 ^z × NZW. H-2 ^d F ₁	z/d	+	+	+	+	+	#	+	#

anti-DNA antibodies would be produced by CD5 B cells, the development of SLE and B-CLL in different members of New Zealand mouse family could be explained by the difference in their predisposition of regulatory abnormalities on the proliferation and differentiation of CD5 B cells.

Based on such assumption, we asked whether CD5 B cells are indeed responsible for anti-DNA antibody production in NZB/W F₁ mice. While young NZB/W F₁ mice spontaneously produce IgM, but not IgG, anti-DNA antibodies, the major isotype of serum anti-DNA antibodies switches from IgM to IgG, beginning at about 6 months of age, the time when SLE begins to develop (Shirai et al. 1991b). This event is associated with changes in the frequency of IgM and IgG producing cells in the spleen. In vitro studies on surface phenotypes of B cells responsible for anti-DNA antibodies showed that while IgM-producing B cells were CD5⁺LP-3 (CD43)⁻, IgG producing cells were CD5⁻LP-3⁺ (Okada et al. 1990).

There are at least two possible interpretations of these findings. First, the IgG anti-DNA antibody-producing cells are derived from conventional B cells. Alternatively, these cells are CD5⁺ B cells that have lost CD5 expression during the process of IgM to IgG isotype switching. There are several events that favor the latter possibility (Shirai et al. 1992). The flow cytometry analysis of spleen cells from NZB/W F₁ mice at varying ages showed that a rapid age-associated decline in the proportion of CD5⁺sIgM⁺ B cells occurs in these mice, beginning at about age 6 months, in association with the generation of CD5⁻sIgM⁻sIgG⁺ B cells (Okada et al. 1990). The most remarkable finding is that this rapid age-associated decrease of CD5 B cells does not occur in H-2-congenic H-2^{d/d} and H-2^{z/z} homozygous NZB/W F₁ mice, that have lost the ability to produce IgG anti-DNA antibodies (Okada et al. 1991). This finding is consistent with the idea that the persistence of CD5 B cells in high frequency in these homozygotes is due to the lack of genetic element (H-2^{d/z}) for differentiation of CD5 B cells (IgM to IgG isotype switching), an event which is probably associated with the loss of CD5 molecules.

Wofsy and Chiang (1987) treated NZB/W F₁ mice by administration of anti-CD4 monoclonal antibody and we treated them with anti-MHC class II antibodies (unpublished observation). What happened in these mice was the decrease in the production of IgG anti-DNA antibodies and, in turn, the increase in the proportion of CD5 B cells. These findings also support the notion that CD5 B cells in NZB/W F₁ mice would undergo maturation in association with IgM to IgG isotype switching and with even a loss of surface CD5 molecules.

More direct evidence was obtained in in vitro culture studies. To investigate the immediate precursor of CD5⁻sIgM⁻sIgG⁺ B cells involved in IgG anti-DNA antibody production, we sorted sIgM⁺ B cells with and without CD5 expression from NZB/W F₁ mice aged 6 months (the time when IgG anti-DNA repertoire begins to generate) and compared their in vitro potential to produce IgG anti-

DNA antibodies. The results showed that IgG anti-DNA antibodies were mainly produced by CD5⁺sIgM⁺ B cells, particularly in the presence of IL-6 (Kanno et al. 1993). As the total IgG synthesis was also promoted by IL-6 to a much greater extent in CD5⁺sIgM⁺ than in CD5⁻sIgM⁺ B cells, the former population of B cells from NZB/W F₁ mice at this age probably contains cells going through the process of maturation, including IgM to IgG isotype switching (IgM/IgG double producers).

We then asked whether there are clonally related IgM and IgG anti-DNA producers in NZB/W F₁ mice aged about 6 months. To answer this question, we established from a single NZB/W F₁ mouse many hybridoma clones producing anti-DNA antibodies and found that this is indeed the case. Sequence analyses of Ig V region genes revealed that while the IgM antibody was coded for by germline V gene, the clonally related IgG counterpart contained many somatic mutations in the V region. Such V gene mutations were associated with affinity maturation (Taki et al. 1992).

There is evidence to suggest that the events such as IgM to IgG isotype switching and somatic mutations in V region genes in association of affinity maturation of anti-DNA antibodies occur under the effects of autoreactive CD4⁺ T cells (Wofsy and Seaman 1985; Sekigawa et al. 1986, 1987). Therefore, together with the data of genetic analyses, we proposed that a combination of particular structures of MHC class II molecules (mixed-haplotype class II molecules) on B cells and TCR (particular repertoires) on T cells may be prerequisite for the interplay between autoreactive T cells and CD5 B cells, resulting in the generation of pathogenic IgG anti-DNA antibodies (Shirai et al. 1987).

All these observations and considerations taken collectively suggest that both autoimmune disease like SLE and B-CLL are the disorders associated with the genetically-predisposed, abnormal regulation of CD5 B cells, one differentiation and one proliferation. The observed high frequency of CD5 B cells and the subsequent development of CD5 B-CLL in H-2^{z/z} homozygous New Zealand mice may probably be explained by at least two mutually unexclusive genetic factors. First, because of the lack of genetic elements for differentiation of CD5 B cells, only signals for proliferation would be operating in CD5 B cells in these mice. Second, a recessive effect of a certain H-2^z-linked defective gene, which may normally control the proliferation of CD5 B cells, is probably involved in a stage of multi-step leukemogenesis, as in the case of tumor suppression gene.

Finally, as B-CLL does not develop in the B10.NZW strain, an H-2-congenic B10 mouse strain bearing the H-2^z haplotype introduced from the NZW strain, B-CLL is also a polygenic disease (Okada et al. 1991). Thus, the model of New Zealand mouse family provides a clue for our better understanding of the mechanism of the correlation between autoimmune disease and B-CLL.

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