

DISEASES CAUSED BY REACTIONS OF T LYMPHOCYTES
TOWARDS INCOMPATIBLE STRUCTURES OF
THE MAJOR HISTOCOMPATIBILITY COMPLEX

VI. Autoantibodies Characteristic of Systemic Lupus Erythematosus
Induced by Abnormal T-B Cell Cooperation Across I-E*‡

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The pathogenic significance of the increased association of systemic lupus erythematosus (SLE) with certain structures of the major histocompatibility complex (MHC) (1-4) is unknown. An experimental model of SLE, in which MHC structures play a defined pathogenic role, is the induction of an SLE-like graft-vs.-host reaction (GVHR) in nonirradiated F₁ mice (5). In this model, the injection of donor T cells and an H-2 incompatibility in the F₁ recipients, regardless of the haplotype, was required for the formation of all autoantibodies studied (6-8). The autoantibody formation during the GVHR is probably the outcome of two consecutive steps. First, alloreactive donor T helper (T_H) cells react against H-2-incompatible B cells and/or macrophages of the F₁ recipient. Thereafter, this reaction, in combination with self-antigen, leads to a positive allogeneic effect that preferentially triggers those F₁ B cells that produce autoantibodies characteristic of SLE (5).

Loci within the I region of H-2, I-A, and I-E code for determinants that control antigen presentation and the interactions of T cells with B cells and macrophages (9). Incompatibilities at I-A and/or I-E cause a strong stimulation in the mixed lymphocyte reaction (MLR) (10, 11) and the GVH splenomegaly assay (12). In the present paper, we report that induction of a GVHR across I-E triggered the formation of autoantibodies characteristic of SLE.

Materials and Methods

Mice. Strains B10.A(2R) and B10.A(4R) were purchased from Olac 1976 Ltd. (Bicester, Oxon, United Kingdom), and [B10.A(2R) × B10.A(4R)]F₁ mice were bred in our animal facilities. Female mice, 8-10 wk old, were used. The H-2 genotypes of the strains of mice used are shown in Table I.

Induction of GVHR. Single-cell suspensions of donor spleen and lymph node cells were prepared as described previously (6). Cell suspensions contained approximately one part lymph-node

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TABLE I
H-2 Genotypes of Strains of Mice Used

Strain	H-2 haplotypes	Alleles at H-2 (sub)regions							
		K	I					S	D
			A	B	J	E	C		
B10.A(2R)	h2	k	k	k	k	k	d	d	b
B10.A(4R)	h4	k	k	b	b	(b)*	b	b	b
[B10.A(2R) × B10.A(4R)]F ₁	h2 × h4	k	k	k/b	k/b	k/(b)*	d/b	d/b	b

* The I-E^b allele does not phenotypically express an E_α chain (13). See Discussion for details.

cells and two parts spleen cells. Nonirradiated F₁ recipient mice were injected intravenously with 50×10^6 viable donor cells on both day 0 and day 7.

Detection of Autoantibodies. Autoantibodies to thymocytes were detected by a complement-dependent cytotoxicity test, and the cytotoxic index (CI) was determined as described (6). ⁵¹Cr-labeled thymocytes from normal [B10.A(2R) × B10.A(4R)]F₁ mice were used as target cells. IgG autoantibodies to erythrocytes were detected by the direct Coombs' test (5). IgG antibodies against nuclear antigens were determined by use of an indirect immunofluorescence technique using a fluorescein-labeled rabbit anti-mouse IgG serum; cryostat sections of mouse liver were used as antigenic substrate as described (7). IgG antibodies reacting exclusively to double-stranded DNA (dsDNA) were determined as described (8).

Results

Untreated B10.A(2R) ($n = 10$), B10.A(4R) ($n = 40$), and [B10.A(2R) × B10.A(4R)]F₁ ($n = 10$) mice served as negative controls. All these mice were bled and tested at 8 and 20 wk of age. None of them had spontaneous autoantibodies to thymocytes, erythrocytes, nuclear antigens, or dsDNA. The administration of B10.A(4R) lymphocytes to F₁ mice induced the formation of autoantibodies against thymocytes, erythrocytes, nuclear antigens, and dsDNA in all of the recipients. In marked contrast, none of the F₁ mice injected with lymphocytes from B10.A(2R) produced autoantibodies to erythrocytes or dsDNA; moreover, autoantibodies to nuclear antigens appeared in just two F₁ recipients, and only one F₁ mouse of the total group had anti-thymocyte antibodies in the serum giving a CI value >30 (Table II).

Discussion

The mainly negative results obtained with the donor B10.A(2R) indicate that the H-2 incompatibilities in the F₁ recipients at the subregions I-B, I-J, I-C, and the S region were too weak to trigger SLE-like autoantibody formation. This finding conforms with the inability of these H-2 (sub)regions to evoke a significant GVH splenomegaly (12) or MLR reactivity (10, 14). In contrast, when B10.A(4R) donor cells were used for the induction of GVHR, autoantibody formation ensued. In this case, the only incompatibility, other than those at I-B, I-J, I-C, and S, against which the B10.A(4R) cells could react, was that determined by the I-E^k subregion of the F₁ recipients (Table I). It is known that the molecular product of the I-E subregion, the E_α chain, has to combine with the E_β chain, coded for by the I-A subregion, to be expressed as a two-chain molecule on the cell surface (13). The crucial point here is that strains of mice possessing a b, f, q, or s allele at I-E do not express the E_α/E_β molecule on the cell surface. One such nonexpressor strain is B10.A(4R) (I-E^b). By

TABLE II
 Formation of SLE-Like Autoantibodies in [B10.A(2R) × B10.A(4R)]F₁ Mice Injected with 10⁸ Parental Strain Lymphocytes

Weeks after first injection of donor cells	Number of F ₁ mice tested	Erythrocytes			Thymocytes			Nuclear antigens			dsDNA		
		Percent at time indicated	Cumulative percent	Mean CI values ± SE of total group	Percent at time indicated*	Cumulative percent*	Mean log titer of positive mice ‡	Percent at time indicated	Cumulative percent	Mean log titer of positive mice ‡	Percent at time indicated	Cumulative percent	Mean log titer of positive mice ‡
F ₁ mice with autoantibodies against													
Donor B10.A(2R)													
1	16	0	0	6 ± 4	0	0	<1	0	0	<1	0	0	<1
5	16	0	0	8 ± 5	6	6	<1	0	0	<1	0	0	<1
9	15	0	0	3 ± 3	7	7	1.0	7	7	1.0	0	0	<1
13	14	0	0	5 ± 2	7	7	2.5	7	7	2.5	0	0	<1
17	12	0	0	3 ± 3	7	7	2.4	17	17	2.4	0	0	<1
Donor B10.A(4R)													
1	16	0	0	5 ± 3	0	0	<1	0	0	<1	0	0	<1
5	15	40	40	44 ± 2	87	93	3.3	100	100	3.3	100	100	1.8
9	12	92	94	38 ± 2	75	93	3.7	100	100	3.7	83	100	2.7
13	9	44	100	49 ± 3	89	93	3.7	100	100	3.7	100	100	2.2
17	9	22	100	42 ± 3	89	100	3.8	100	100	3.8	100	100	2.1

* Only those F₁ mice whose sera gave a CI > 30 were considered to be positive, because normal mice may have serum antibodies to thymocytes, which give CI values up to 30 when compared with the normal mouse serum used as reference.

‡ All SE values of log titers in this column were ≤ 0.2.

contrast, strain B10.A(2R) (I-E^k) does express the E_α/E_β molecule (13). Hence, T cells of B10.A(2R) were not exposed to an I-E incompatibility on B cells and macrophages of [B10.A(2R) × B10.A(4R)]F₁ recipient mice, whereas the T cells of B10.A(4R) could react against the E_α^k/E_β^k molecule expressed by such F₁ mice (9, 13). This one-way reaction between lymphoid cells of these strains explains (9) the previous observations that mixed-lymphocyte reactivity (10, 14) and GVH splenomegaly (12) occur only in the direction B10.A(4R) anti-B10.A(2R) and not in the opposite direction. The same unidirectionality was found in the present study on the induction by GVHR of SLE-like autoantibodies (Table II).

Thus, our results indicate that an incompatibility at I-E alone provided the stimulus that led to the massive formation of autoantibodies characteristic of SLE. The observed formation of high titers of IgG antibodies to dsDNA (Table II) is especially remarkable because dsDNA has proved to be a very poor immunogen in conventional immunization procedures (15). In all likelihood, the initial reaction leading to autoantibody formation was made by the alloreactive T_H cells of the donor B10.A(4R) against the E_α^k/E_β^k molecule present on F₁ macrophages and/or B cells.

An intriguing aspect of these findings is that I-E appears to be the murine analogue of HLA-D/DR (16, 17). Therefore, our findings are of interest with respect to the increased frequency of certain HLA-DR alleles in patients with SLE (1-4). It has been proposed that the formation of autoantibodies in SLE might be due to a GVH-like abnormal cooperation between autologous T and B cells (5). The postulated role of HLA-D/DR in this process would be to combine with etiologic agents, such as viruses or drugs, and then provide an immunogenic stimulus to autologous T_H cells. Thereafter, the activated T_H cells might, in a GVH-like fashion, trigger the normally existing autoreactive B cells involved in SLE (5). Recent studies performed in mice with the SLE-inducing drug diphenylhydantoin are consistent with this concept (18). Conceivably, a given etiologic agent becomes strongly immunogenic for T_H cells only in combination with one or a few alleles of HLA-D/DR, or closely linked loci. This concept might explain why some groups of patients with idiopathic SLE show an increased frequency with HLA-DR3 (1, 2), whereas in others, possibly exposed to a different etiologic agent, there seems to be an association with HLA-DR2 (1, 3). When patients with hydralazine-induced SLE were studied, an increased association with HLA-DR4 was found (4). Thus, regardless of the variable etiology of SLE, these are products of the same locus, HLA-DR, that appear to be involved in the pathogenesis of the disease. The involvement in a well-defined model of SLE of the analogous murine locus, I-E, may help to understand these intriguing pathogenic relationships.

Summary

By induction of a suitable graft-vs-host reaction (GVHR) in H-2-different F₁ mice, one can induce the production of autoantibodies characteristic of systemic lupus erythematosus (SLE). The purpose of the present study was to define the intra-H-2 differences in the F₁ recipients that are capable of triggering this process. A GVHR was induced in [B10.A(2R) × B10.A(4R)]F₁ mice by injecting 10⁸ lymphocytes from either parental strain. Whereas the donor B10.A(4R) induced a massive formation of autoantibodies to thymocytes, erythrocytes, nuclear antigens, and double-stranded DNA, the donor B10.A(2R) failed to do so. The intra-H-2 genetics of these two parent →F₁ combinations are such that the observed autoantibody formation after the

injection of B10.A(4R) T cells must have been triggered exclusively by the incompatible I-E^k subregion of the [B10.A(2R) × B10.A(4R)]F₁ recipients. Because I-E appears to be the murine analogue of HLA-D/DR, this finding is of interest with respect to the increased frequency of certain HLA-DR alleles in SLE patients, as discussed.

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