AUTOANTIBODIES IN CHRONIC GRAFT VERSUS HOST RESULT FROM COGNATE T-B INTERACTIONS

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Systemic lupus erythematosus (SLE), in mice as in humans, is characterized by a wide spectrum of autoantibodies mainly directed at cellular antigens (1, 2). T cells are apparently required for the induction of such autoantibodies, but their exact role in spontaneous disease remains to be defined (3, 4). In chronic graft-versus-host (GVH)[1] disease, an SLE-like syndrome can be induced in normal mice by transfer of allogeneic T cells. It is thought that the allohelper T cells of the donor react against incompatible Ia structures of the host and generate excessive help, which activates a subpopulation of B cells in the host that is self reactive (5–7). Such a GVH reaction occurs between B6 (or its Igh allotype congenic strain B6.C20) and bm12 mice through the recognition of the mutant bm12 Ia by the B6 (or B6.C20) cells, and vice versa (6; and Morris, S. C., P. L. Cohen, and R. A. Eisenberg, manuscript submitted for publication). A syndrome that closely resembles SLE is induced, with autoantibodies to erythrocytes, chromatin, nuclear antigens, and to a more limited degree, double-stranded DNA.

Activation of resting B cells to proliferate and differentiate into antibody-producing cells occurs through the binding of antigen by the B cell surface Ig receptor and the participation of T cell help. Previous studies have established that Th cells can support antigen-specific B cell responses through two distinct pathways (8–13). Cognate help results from direct T-B contact, while noncognate or bystander help is mediated through factors released from activated T cells that can act at a distance upon antigen-activated B cells. Since chronic GVH results from the stimulation of alloreactive T helper cells (14), and their inappropriate collaboration with the host B cells (5, 15), we investigated whether the donor T cells recognized foreign Ia directly on the B cell stimulated to make autoantibodies. Double-congenic chimeras, containing two sets of B cells that differed at both Ia and Igh allotype, were used to determine whether the autoantibody forming cells were specifically stimulated by the autoreactive T cells and/or if a bystander effect was occurring. The isotype and allotype of the Coombs antibodies were determined, as was the allotype of the IgG2a

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[1] *Abbreviations used in this paper:* BBS, borate buffered saline; EDF, equivalent dilution factors; GVH, graft-versus-host; MRBC, mouse red blood cells.

and IgM antichromatin antibodies. We found that autoantibody production resulted almost entirely from cognate recognition (direct T-B contact).

Materials and Methods

Experimental Animals. C57BL/6Kh (B6: H-2^b, Igh^b), B6.C-H-2^{bm12} (bm12: H-2^{bm12}, Igh^b), B6.C20 (H-2^b, Igh^a), and (B6.C20 × bm12)F_1 mice were maintained in our breeding facility. The B6 and bm12 mice were originally obtained from Roger Melvold (Northwestern University, Chicago, IL), and the B6.C20 mice were from Gayle Bosma (Institute for Cancer Research, Philadelphia, PA). All mice used in this study were female. Mice were entered into two separate experiments at different times. As the results with these two cohorts were entirely comparable, pooled data for all mice are presented.

Antibodies (Table I). Monoclonal anti-isotype/allotype antibodies or control antibodies were either obtained commercially, or isolated from ascites or tissue culture supernatants (HB100, TIB148, 2E.6, 4F3, and MPC11). Ascites was produced in mice with the appropriate H-2 and Igh allotypes.

Production of Chimeras (Table II). Recipient (B6.C20 × bm12)F_1 mice, 2–5 mo old, were given neomycin sulfate in their drinking water for 48 h before irradiation and for a minimum of 8 wk thereafter. They received 850–900 rad in a Gamma-Cell 40 apparatus (Atomic Energy of Canada, Ltd., Ottawa, Ontario, Canada) on the morning of bone marrow transfer. The mice were reconstituted intravenously with 1.25–1.5 × 10^7 F_1 or parental bone marrow cells, obtained from the tibias and femurs of 2–7-mo-old mice and treated with monoclonal anti-Thy-1.2 (NEN Research Products, Boston, MA) plus complement. 4–5 mo after bone marrow repopulation, recipients were checked for chimerism by flow cytometry of their peripheral

TABLE I
Monoclonal Reagents

Name	Source (reference)	Species	Isotype	Antibody specificity
HB100 (Bet 1)*	ATCC (16)	Rat	IgG1	IgM^a
AF6-78.25.2	Stall (17)	Mouse	IgG1	IgM^b
20-9.10	Oi (18)	Mouse	IgG2a	$IgG1^a$
412-14.4	Oi	Mouse	IgG1	$IgG1^b$
TIB148 (Ig(1a)8.3)	ATCC (18)	Mouse	IgG2a	$IgG2a^a$
Ig(1b)3.1.5.9	Oi (18,19)	Mouse	IgG1	$IgG2a^b$
21-48.1	Oi	Mouse	IgG2a	$IgG2b^a$
412-72.1	Oi	Mouse	IgG1	$IgG2b^b$
2E.6	ATCC	Rat	IgG1	IgG3
D3-137.5	Tonkonogy	Mouse	IgG2a	$I\text{-}A^b$
MOPC21	Litton	Mouse	$IgG1^a$	–‡
MOPC245	Potter	Mouse	$IgG1^b$	–
HB63 (1-29-7)	ATCC (20)	Mouse	$IgG2a^a$	–
4F3	Ross	Mouse	$IgG2a^a$	–
CBPC101	Potter	Mouse	$IgG2a^b$	–
MPC11	ATCC (21)	Mouse	$IgG2b^a$	–
MOPC195	Litton	Mouse	$IgG2b^a$	–
BPC4	Potter	Mouse	$IgG2b^b$	–
J606	Litton	Mouse	IgG3	–
FLOPC21	Litton	Mouse	IgG3	–
TEPC183	Litton	Mouse	IgM^a	–
CBPC112	Potter	Mouse	IgM^b	–

* HB100 was specific in our ELISAs and in our Coombs assay, but was not able to be used by us for staining.

‡ Monoclonal used as antigen for standardizations.

TABLE II
GVH Chimeric Mice

Group	Bone marrow donor	B cell phenotype	Spleen cell graft
I	(B6.C20 × bm12)F_1	Igh^a,H-$2^{b/bm12}$ and Igh^b,H-$2^{b/bm12}$	bm12
II	"	"	B6
III	B6.C20 and bm12	Igh^a,H-2^b and Igh^b,H-2^{bm12}	bm12
IV	"	"	B6
V	(B6.C20 × bm12)F_1	Igh^a,H-$2^{b/bm12}$ and Igh^b,H-$2^{b/bm12}$	(B6.C20 × bm12)F_1
VI	B6.C20 and bm12	Igh^a,H-2^b and Igh^b,H-2^{bm12}	(B6.C20 × bm12)F_1

blood lymphocytes. Briefly, 250 μl of peripheral blood was collected in heparin, diluted with 250 μl of PBS, and layered over 500 μl of Lympholyte M (Cedarlane Laboratories, Hornby, Ontario). After centrifugation and washing, the cells were stained for I-A^b with biotinylated mAb D3-137.5 (IgG2a), and for IgM^b with mAb AF6-78.25.2 (IgG1). For two-color analysis, streptavidin-phycoerythrin (Becton Dickinson & Co., Mountain View, CA) and fluorescein-labeled goat anti-mouse IgG1 (Southern Biotechnology Associates, Birmingham, AL) were used as the second-step reagents.

Induction of GVH Disease (Table II). GVH disease was induced in chimeric mice by injecting intraperitoneally 10^8 viable parental spleen cells from 5–9-mo-old donors. F_1 spleen cell injected mice served as controls. Single cell suspensions were prepared by pressing the spleens through a mesh wire screen into HBSS. The cells were washed three times in HBSS before injection.

Follow-up of Mice. Mice were bled at intervals of 2–5 wk by retro-orbital sinus puncture under ether anesthesia. 50 μl of blood, diluted in Alsever's solution (2.05% dextrose, 0.43% NaCl, and 0.8% sodium citrate), was taken for the determination of antierythrocyte antibodies (Coombs). Additional blood was clotted, and the serum separated and stored at −20°C for future testing.

Determination of Anti-Mouse Erythrocyte (Coombs) Antibodies. The direct Coombs test for the detection of bound antierythrocyte antibodies was performed with a 1% suspension of washed mouse erythrocytes in PBS containing 1% heat-inactivated FCS. 50 μl of rabbit anti-mouse γ (heavy and light chain specific), diluted 1:20, were placed in a round-bottomed polystyrene microtiter plate (Dynatech Laboratories, Inc., Alexandria, VA) and 50 μl of the erythrocyte suspension was added. As a negative control, 50 μl of buffer alone was mixed with the erythrocytes. The microtiter plates were scored for hemagglutination on a scale of 0 to 4+.

Determination of the Isotype and Allotype of the Coombs Antibodies. Purified mAbs (see Table I) specific for either the a or b allotype of the isotypes γ1, γ2a, γ2b, or IgM, as well as a mAb specific for γ3 (γ3 is not polymorphic in inbred mice), were coated on sheep erythrocytes (SRBC) by the $CrCl_3$ method (22). Monoclonal control proteins of the a or b allotype for each isotype were also coated on SRBC and used to confirm the specificity of the SRBC coated with the anti-isotype/allotype reagents. To validate the assay, erythrocytes were resuspended to 0.5% in PBS containing 1% heat-inactivated FCS, and equal mixtures of the anti-isotype/allotype protein-coated SRBC were mixed with monoclonal control protein-coated SRBC and scored for hemagglutination. The isotype and allotype of the GVH anti-MRBC antibody were then determined by mixing 50 μl of a 0.5% suspension of Coombs-positive erythrocytes with 50 μl of antibody-coated SRBC in a polystyrene microtiter plate. The results were scored for hemagglutination on a scale of 0 to 4+.

Determination of Serum $IgG2b^b$ Levels. A hemagglutination assay for the estimation of relative serum levels of $IgG2b^b$ was performed using SRBC coated with anti-$IgG2b^b$-purified mAb as described above. 50 μl of a 1% suspension of antibody-coated SRBC was mixed with 50 μl of serum diluted serially twofold from 1:1,000 to 1:16,000 in a polystyrene microtiter plate. The results were scored for hemagglutination.

ELISA of IgG and IgM Antichromatin. Chromatin antigen was purified from chicken erythrocyte nuclei (23). Polyvinylchloride microtiter plates (Dynatech Laboratories, Inc.) were coated with 100 μl of a 10 μg/ml chromatin solution in borate-buffered saline (BBS) for 5 h

at room temperature. The plates were then washed with BBS and incubated with 200 μl of coating buffer (BBS, 0.5% normal goat serum, 0.4% Tween 80, 0.5% BSA, and 0.1% NaN_3) for 2 h at room temperature. After washing the plates, serum samples diluted 1:500 in coating buffer were added. The plates were incubated overnight at 4°C. The following day, the plates were washed and a 1/4,000 dilution of biotinylated affinity-purified goat anti-mouse IgG (pFc' specific) or a 1/500 dilution of goat anti-mouse IgM (heavy chain specific) were added for 1 h at 4°C. The plates were then washed and incubated with avidin-alkaline phosphatase (Zymed Laboratories, So. San Francisco, CA) for 1 h at 4°C. After washing, paranitrophenyl phosphate substrate (Sigma Chemical Co., St. Louis, MO) was added in 0.01 M diethanolamine, pH 9.8. The plates were read at multiple time points with an automated micro-ELISA reader (Dynatech Laboratories, Inc.). The anti-IgG and anti-IgM reagents were standardized to each other, and the results from individual assays were standardized against a high titer MRL/Mp-*lpr/lpr* mouse serum, and equivalent dilution factors (EDF) were calculated according to the following formula: (Dilution of standard reference sera that gives the equivalent OD of the test serum) × 10^6 (24). Sera were considered positive for antichromatin antibodies if they exhibited an EDF value ≥15.

ELISA of Allotype-specific Antichromatin. The allotype of IgG2a and IgM antichromatin antibodies was quantitated by an assay similar to that for IgG and IgM antichromatin, except that the serum was diluted 1:250 and the assay was developed with standardized biotinylated monoclonal antiallotype reagents (anti-$IgG2a^a$, anti-$IgG2a^b$, anti-IgM^a, or anti-IgM^b). The relative amounts of a and b allotype IgG2a antichromatin antibodies in the test sera were determined by comparison with standard sera from a MRL/Mp-*lpr/lpr* mouse (Igh^j, equivalent to Igh^a) and from a C57BL/6-*lpr/lpr* mouse (Igh^b). These sera were chosen to have equivalent amounts of IgG2a antichromatin antibodies, such that the results of the $IgG2a^a$ and $IgG2a^b$ assay could be expressed as comparable EDF. In the IgM^a and IgM^b allotype-specific assay, the C57BL/6-*lpr/lpr* and the MRL/Mp-*lpr/lpr* reference sera were validated in every assay by developing with goat anti-mouse IgM. These results were also expressed as EDF.

ELISA of Total Serum $IgG2a^a$ and $IgG2a^b$. Total serum IgG2a of the a or b allotype was measured by coating the microtiter plates with rat anti-mouse κ chain mAb (HB58, ATCC) at a concentration of 0.1 μg/well. The plates were then washed and incubated with coating buffer. After washing the plates, the sera were added, diluted to 1:10,000 and 1:100,000, for an overnight incubation. The plates were washed, and biotinylated monoclonal anti-$IgG2a^a$ and anti-$IgG2a^b$ reagents were added in the presence of excess mouse IgG myeloma ascites of the opposite allotype (e.g., anti-$IgG2a^a$ was mixed with b allotype ascites) in order to block free binding sites of the rat anti-mouse κ antibody. After incubating the biotinylated reagent for 2 h the plates were washed, and avidin-alkaline phosphatase was added for 2 h, followed by paranitrophenyl phosphate substrate. The results from individual assays were standardized with purified monoclonal $IgG2a^a$ or $IgG2a^b$ proteins, and the results were expressed as micrograms per milliliter.

ELISA of Total Serum IgM^a and IgM^b. Serum levels of IgM^a and IgM^b were measured by a modification of the serum IgG2a allotype ELISA. Microtiter plate wells were coated with affinity-purified goat anti-mouse IgM. The remainder of the assay was the same except that the sera were tested at dilutions of 1:2,000 and 1:4,000, and the assay was developed with biotinylated monoclonal anti-IgM (a or b allotype specific) reagents without excess ascites being present. The results were standardized with IgM^a and IgM^b myeloma proteins, and the results were expressed as micrograms per milliliter.

Results

Generation of Chimeras. To study whether self-reactive host B cells are activated by direct interaction with alloreactive T cells or by nonspecific bystander effects, we generated two types of chimeras. Specifically, (B6.C20 × bm12)F_1 mice, heterozygous for Ia (Ia^b/Ia^{bm12}) and for Igh allotype (Igh^a/Igh^b), were irradiated and reconstituted with either an equal mixture of bm12 and B6.C20 marrow cells ("double-

parental chimeras"); or with F_1 marrow ("control chimeras"). Individual B cells in the double-parental chimeras should express either Igh^b and Ia^{bm12}, or Igh^a and Ia^b; while B cells of the control chimeras should express either Igh^a or Igh^b together with Ia^b/Ia^{bm12}. To verify appropriate bone marrow reconstitution, all of the double-parental chimeras and some of the control chimeras were subjected to two-color immunofluorescence flow cytometric analysis of cell surface IgM^b and $I\text{-}A^b$ expression on their PBL. Double-parental chimeras PBL showed a mean percentage of double staining for IgM^b and Ia^b of only 1.9% ± 0.2 (n = 35), which is no different (p = 0.29) than that seen with a control mixture of equal numbers of PBL from bm12 and B6.C20 mice, 1.2% ± 0.4 (n = 4). These results demonstrated that double-parental chimeras were indeed reconstituted with a mixture of parental cells, and that the host (F_1) B cells did not recover significantly. Control (F_1 reconstituted) chimeras, in contrast, had 9.9% ± 1.2 (n = 11) double staining for IgM^b and Ia^b.

Induction of a Chronic GVH Syndrome. Double-parental chimeras (groups III, IV, and VI) and F_1-reconstituted chimeras (groups I, II, and V) were each injected with 10^8 B6, bm12 or (B6.C20 × bm12)F_1 spleen cells (Table II). A chronic GVH syndrome was evident in those chimeras that received B6 or bm12 spleen cells (groups I–IV), as indicated by accelerated 50% mortality by 9–16 wk (Fig. 1), antichromatin and Coombs autoantibodies peaking at 5–9 wk (see below), and transient ascites not associated with proteinuria (data not shown).

Antierythrocyte Antibodies. As shown in Table III, the numbers of mice that were Coombs-positive ranged from 25% of the mice in group I to 100% of the mice in group IV. All samples found to be direct Coombs-positive by hemagglutination were then screened for both the isotype and allotype of their anti-MRBC antibodies. IgM and all four IgG subclasses of anti-MRBC antibodies appeared without any evidence for isotype preference. Not all Coombs-positive samples were able to be typed for isotype and allotype.

Table IV compiles the allotypes of the Coombs antibodies from all weeks tested. Only a allotype was detected in Coombs-positive mice in group II and in group III. In contrast, group IV mice had predominantly b allotype anti-MRBC antibodies, although three of these mice had $IgG2a^a$ anti-MRBC antibodies in several bleeds, as well as b allotype anti-MRBC antibodies of various isotypes.

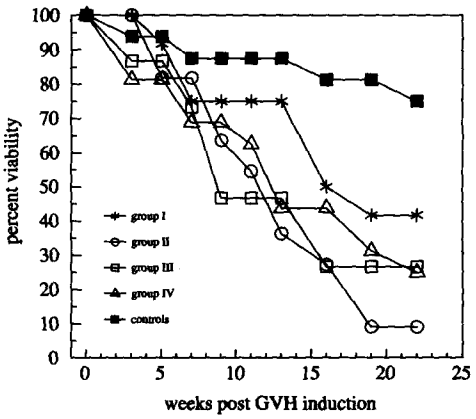

FIGURE 1. Survival of GVH chimera mice. GVH disease was induced in chimeras by injection of either bm12 or B6 spleen cells. A mortality of 50% occurred in the four experimental groups between weeks 9 and 16. The n listed represents the total number of mice that started in each group (Table III). Groups I (*) n = 12, group II (○) n = 11, group III (□) n = 15, and group IV (△) n = 16. The two control groups (■), group V (n = 7), and group VI (n = 9) were pooled.

TABLE III
Antierythrocyte Antibodies in Chimeric GVH Mice

Group	*n*	Coombs-positive	Isotype detected*				
			γ1	γ2a	γ2b	γ3	μ
I bm12→(B6.C20 × bm12)F_1	12	3	0	0	0	1	0
II B6→(B6.C20 × bm12)F_1	11	6	4	1	0	5	0
III bm12→B6.C20 + bm12	13	10	7	3	0	4	0
IV B6→B6.C20 + bm12	13	13	5	8	8	11	12
V (B6.C20 × bm12)F_1→(B6.C20 × bm12)F_1	6	0	–	–	–	–	–
VI (B6.C20 × bm12)F_1→B6.C20 + bm12	9	0	–	–	–	–	–

* Every Coombs-positive bleed for each mouse was tested with the panel of anti-allotype/isotype-specific mAb-coated SRBC. Numbers presented represent the number of mice in each group for which a particular isotype occurred at least once.

Antichromatin Antibodies. The serum levels of both IgG and IgM antichromatin autoantibodies were assessed by ELISA (Table V). 44 of the 49 mice in groups I–IV were positive for IgG antichromatin autoantibodies, and 38 were positive for IgM antichromatin autoantibodies. In the mice with both IgG and IgM antichromatin antibodies, the initial IgM and IgG response generally coincided, although IgM tended to peak early and then diminish, while IgG continued to increase. The peak titers of IgG antichromatin antibodies were higher than those of IgM. As we have found in other GVH mice, IgG2a was the predominant subclass of the IgG antichromatin responses (data not shown).

Allotype of the Antichromatin Autoantibodies. The amounts of a and b allotype IgG2a antichromatin autoantibodies were quantitated for the mice in groups I–IV at all time points. Fig. 2 shows the titers of the $IgG2a^a$ and $IgG2a^b$ antichromatin antibodies for each mouse at its peak IgG antichromatin response. Group III mice were only positive for $IgG2a^a$ and group IV for $IgG2a^b$ antichromatin antibodies. This pattern held true throughout the course of the experiment (data not shown). In groups I and II, both allotypes were seen, although a preference for $IgG2a^b$ antichromatin antibodies was evident, since all of the mice except two were to the left of the equivalence line ($IgG2a^b$ equals $IgG2a^a$).

Fig. 3 shows the results for the IgM antichromatin allotypes, which were determined at the peak IgM titer week in IgM antichromatin-positive mice. The IgM

TABLE IV
Coombs Allotype in Chimeric GVH Mice

Group	*n*	Allotype*	
		a	b
I bm12→(B6.C20 × bm12)F_1	12	0	0
II B6→(B6.C20 × bm12)F_1	11	10	0
III bm12→B6.C20 + bm12	13	14	0
IV B6→B6.C20 + bm12	13	8	72

* Every Coombs-positive bleed for each mouse was tested with the panel of anti-allotype/isotype-specific mAb-coated SRBC. Numbers presented represent the sums of positive reactions of the a and b allotypes, respectively.

TABLE V

IgG and IgM Antichromatin Antibodies in Chimeric GVH Mice

Group	n	Number positive*		Titer‡	
		IgG	IgM	IgG	IgM
I bm12→(B6.C20 × bm12)F_1	12	12	12	102	52
II B6→(B6.C20 × bm12)F_1	11	7	5	25	19
III bm12→B6.C20 + bm12	13	13	11	110	35
IV B6→B6.C20 + bm12	13	12	10	77	20
V (B6.C20 × bm12)F_1→(B6.C20 × bm12)F_1	6	2	1	13	11
VI (B6.C20 × bm12)F_1→B6.C20 + bm12	9	2	0	10	7

* Mice were positive for antichromatin antibodies if the EDF was ≥15.

‡ Geometric means of the peak EDF for all mice in the group. Standard errors (×/÷) were 1.1–1.3.

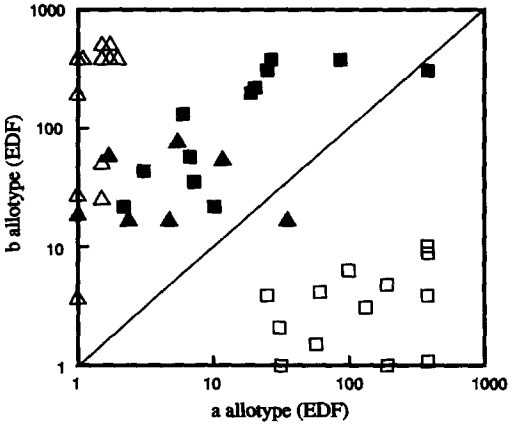

FIGURE 2. Allotypes of IgG2a antichromatin antibodies in chimeric GVH mice. The levels of $IgG2a^a$ and $IgG2a^b$ antichromatin antibodies, for the peak IgG antichromatin week, are shown for the mice that were positive for IgG antichromatin antibodies (EDF ≥15). Group I (■), group II (▲), group III (□), and group IV (△).

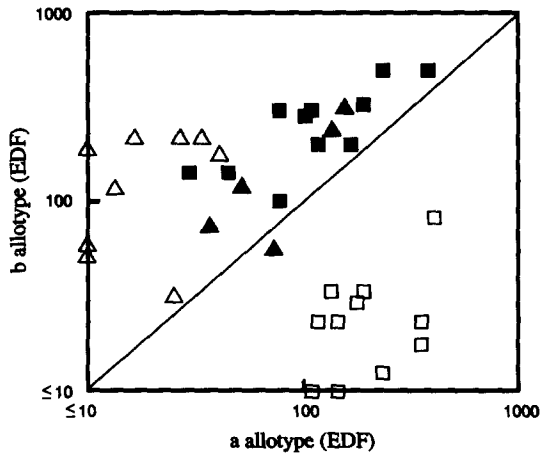

FIGURE 3. Allotypes of IgM antichromatin antibodies in chimeric GVH mice. The levels of IgM^a and IgM^b antichromatin antibodies, for the peak IgM antichromatin week, are shown for those mice that had an EDF of ≥20 for IgM antichromatin antibodies. Group I (■), group II (▲), group III (□), and group IV (△).

antibodies in group III were strongly skewed to the a allotype, while those in group IV were skewed to the b allotype. In contrast to the IgG2a results, however, IgM anti-chromatin antibodies of the b and a allotypes were present in groups III and IV, respectively, albeit in low titers. In the control chimeras (groups I and II) a slight

TABLE VI

Total Serum IgG2a Allotype in IgG Antichromatin-positive Chimeric GVH Mice

Group	n	Prebleed		Peak*	
		Igh^a	Igh^b	Igh^a	Igh^b
I	12	274‡	500	378	659
II	7	341	667	335	682
III	13	504	610	808	141
IV	12	671	659	105	1,214

* Results shown are from bleeds with highest IgG anti-chromatin titers (see Fig. 2).

‡ Arithmetic mean (μg/ml).

preference for IgM antichromatin antibodies of the b allotype was seen, since data from all of the mice, except one, were slightly to the left of the equivalence line (IgM^a equals IgM^b).

Total Serum Allotype Levels of IgG2a and IgM. The total serum levels of both IgG2a and IgM were determined for the a and b allotype. Table VI summarizes the data for total serum IgG2a for both allotypes in the same bleeds in which IgG2a antichromatin allotypes were measured for IgG antichromatin-positive mice. In the double-parental chimeras (groups III and IV), the prebleed levels of $IgG2a^a$ and $IgG2a^b$ were comparable. At the peak titer week, group III mice showed an increase in $IgG2a^a$ and a decrease in $IgG2a^b$ as compared with their prebleed levels. In contrast, group IV mice showed an increase in $IgG2a^b$ and a decrease in $IgG2a^a$.

Table VII shows similar data for the IgM allotypes. In the double-parental chimeras, groups III and IV, the prebleed levels of IgM^a and IgM^b were comparable. At the peak titer week, group III mice showed a significant increase in IgM^a ($p < 0.0001$) and an insignificant decrease in IgM^b ($p = 0.53$), as compared with their prebleed levels. Group IV mice showed reciprocal results.

The mean decrease in $IgG2a^b$ in group III and in $IgG2a^a$ in group IV reflected a striking allotype-specific suppression of IgG2a as early as the first bleed after GVH induction (week 3) in the double-parental chimeras (Table VIII). All of the mice in group III were suppressed for their $IgG2a^b$ by at least 50% of the prebleed levels. 3 of the 13 mice eventually showed a late recovery of their $IgG2a^b$ levels at a time

TABLE VII

Total Serum IgM Allotypes in IgM Antichromatin-positive Double-Chimeric GVH Mice

Group	n	Prebleed		Peak*	
		Igh^a	Igh^b	Igh^a	Igh^b
III	10	1,016‡	1,171	2,282	980
IV	8	915	1,084	996	2,271

* Results shown are from bleeds with highest IgM antichromatin titers (see Fig. 3).

‡ Arithmetic mean (μg/ml).

TABLE VIII

Allotype-specific Suppression of Total Serum IgG2a in Chimeric GVH Mice

Group	*n*	Suppression* ≥90% IgG2a^a	≥90% IgG2a^b	≥50%, but <90% IgG2a^a	≥50%, but <90% IgG2a^b
I bm12→(B6.C20 × bm12)F_1	12	0	0	0	0
II B6→(B6.C20 × bm12)F_1	11	0	0	0	0
III bm12→B6.C20 + bm12	13	0	11‡	0	2§
IV B6→B6.C20 + bm12	13	10‡	0	3§	0

* All mice before GVH induction had IgG2a of both allotypes. Suppression occurred by the first bleed after GVH induction.

‡ Of these 21 strongly suppressed mice, eight recovered.

§ Of these five partially suppressed mice, four recovered.

when most of the other mice had already died. Group IV mice showed a reciprocal suppression of IgG2a^a. Of additional note, 10 of 12 of the group III mice studied by a hemagglutination assay showed a two- to fourfold reduction in IgG2b^b from their prebleed levels. This contrasts with four mice that were tested from group IV and that showed a similar increase in their serum levels of IgG2b^b.

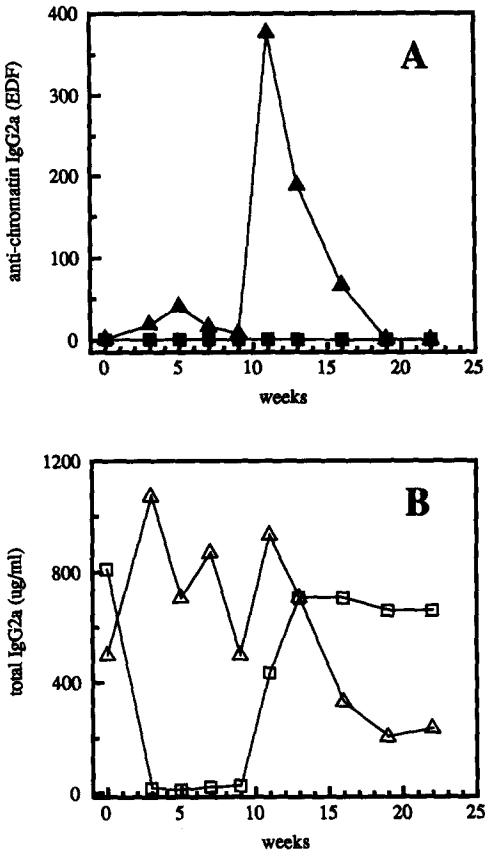

FIGURE 4. Allotype of total serum IgG2a and IgG2a antichromatin antibodies in an individual chimeric GVH mouse. The antichromatin and total IgG2a antibodies are shown for a (B6.C20 + bm12) chimera that received B6 spleen cells (group IV). IgG2a antichromatin antibodies were only of the b allotype and peaked at 11 wk (*A*). Total levels of IgG2a^a became undetectable at 3 wk, but returned towards normal after 9 wk (*B*). Antichromatin IgG2a^a (■), antichromatin IgG2a^b (▲), total IgG2a^b (□), and total IgG2a^b (△).

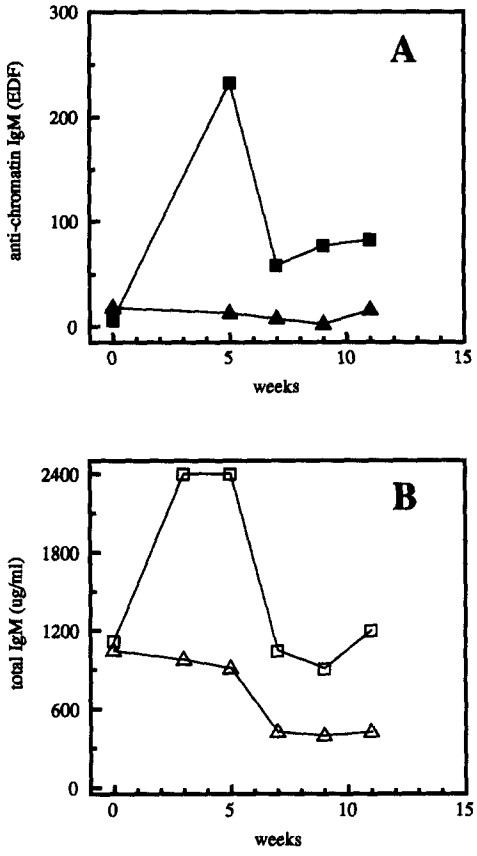

FIGURE 5. Allotype of total serum IgM and IgM antichromatin antibodies in an individual chimeric GVH mouse. The antichromatin and total IgM antibodies are shown for a (B6.C20 + bm12) chimera that received bm12 spleen cells (group III). IgM antichromatin antibodies were only of the a allotype and peaked at week 5 (*A*). Total serum IgM^a showed an early increase (at 3 wk), while IgM^b decreased somewhat (*B*). Antichromatin IgM^a (■), antichromatin IgM^b (▲), total IgM^a (□), and total IgM^b (△).

Figs. 4 and 5 show serial allotype determinations in representative individual double-parental chimeras. A double-parental chimera that received B6 spleen cells (group IV) had $IgG2a^b$ antichromatin antibodies (Fig. 4 *A*). Total levels of the $IgG2a^a$ were totally suppressed at 3 wk, but returned during the peak IgG2a antichromatin response, even though the antichromatin antibodies remained entirely $IgG2a^b$ (Fig. 4 *B*). A double-parental chimera that received bm12 spleen cells (group III) showed IgM^a antichromatin antibodies (Fig. 5 *A*), while total levels of IgM^a increased and IgM^b decreased slightly (Fig. 5 *B*).

Discussion

Chronic GVH results from the administration of alloreactive donor T cells that recognize host Ia and cause the selective stimulation of B cells that produce autoantibodies (5–7). In the current work we have investigated whether the donor T cells must directly recognize foreign Ia on the B cells that are stimulated to make autoantibodies (cognate interaction), or whether such T cells can be activated by Ia and then deliver sufficient help through soluble factors (lymphokines) that would interact with B cells that bear the foreign Ia as well as those that do not. Using double-parental chimeras, differing at both Ia and Igh allotype, we were able to distinguish such

cognate help from bystander interaction in an in vivo situation. Although we transferred whole spleen cell populations to initiate the GVH reaction, the donor B cells were irrelevant, as they do not participate in this syndrome (24a).

Our results strongly indicate that the cognate model of T-B interaction is favored for production of autoantibodies in chronic GVH. Specifically, for both antierythrocyte (Coombs) and antichromatin systems, the autoantibodies were overwhelmingly of the allotypes that would result from cognate T-B collaboration. That is, double-parental chimeras that received bm12 spleen cells made autoantibodies of the Igh^a allotype, presumably as a result of the interaction of the bm12 T cells with the Ia^b/Igh^a host B cells of B6.C20 origin. Conversely, recipients of B6 spleen cells made Igh^b autoantibodies, indicating a cognate interaction between B6 T cells and the Ia^{bm12}/Igh^b host B cells of bm12 origin. Cognate preference for IgG2a antichromatin autoantibodies was absolute, while antichromatin of the IgM isotype showed low level bystander induction along with evidence of strong cognate interaction. We could not test additional isotypes because of limitations of affinity of the antiallotype reagents; however, IgG2a was the main isotype for the antichromatin response in these mice, as it is in other SLE mice (24). The Coombs antibody appeared in multiple isotypes, with no discernable pattern of isotype progression. Nevertheless, in the vast majority of cases, the antibody allotype indicated cognate T-B interaction for each isotype. The exceptions were three mice that received bm12 spleen cells and yet showed $IgG2a^a$ antierythrocyte antibodies, along with a variety of b allotype Coombs antibodies. Although this may indicate an occasional induction of autoantibodies by bystander mechanism, it is also possible that low level recovery of the recipients' endogenous (B6.C20 × bm12)F_1 bone marrow may have permitted a allotype autoantibodies through cognate interaction. Overall, then, our data indicate that the Coombs and antichromatin antibodies in the GVH system are mainly produced through cognate T-B collaboration. We cannot rule out an additional weak bystander effect in some cases, particularly for the IgM isotype.

An additional interesting finding was a relative preference for b-allotype antichromatin antibodies of both the IgG2a and IgM isotypes in the GVH chimeras reconstituted with F_1 bone marrow. This skewing probably results from two separate mechanisms, which we have explored in detail elsewhere (24a). First, the donor T cells in both groups I and II were themselves from animals of b allotype, and therefore would tend to suppress the B cells of the a allotype in the recipient. This would not be expected to be a major factor, since such suppression of the a allotype by b allotype donor cells is weak, in comparison with suppression of b allotype by a allotype donor cells (25). Second, and probably more important, is a general favoring of the b allotype for antichromatin antibodies that we have seen in allotype-heterozygous spontaneous SLE mice (26; and Fisher, C. L., S. C. Morris, P. L. Cohen, and R. A. Eisenberg, manuscript in preparation), and which we have also documented in the GVH system. Although such b allotype skewing may have contributed to the results seen in group IV double-parental chimeras, the striking a allotype preference for autoantibodies in group III double-parental chimeras is counter to this tendency and confirms our interpretation of cognate T-B interaction. Interestingly, the Coombs antibodies in the group II chimeras were entirely of the a allotype, but there were too few mice that responded in this group to draw any firm conclusions.

A particularly surprising result was that suppression of the IgG2a from the non-

cognately recognized B cells occurred in all of the double parental chimeras by the first bleed (week 3) after GVH induction. This suppression was at least 50% of the prebleed level of IgG2a, but was usually >90% of the prebleed, while the IgG2a from the cognately recognized B cells usually showed a reciprocal increase. 8 of the 26 mice in the double-parental chimeras showed a late recovery of their suppressed IgG2a allotype while antichromatin antibodies were still present, but the allotype of these autoantibodies remained only of the cognate allotype. This allotype-specific suppression is probably not comparable to allotype suppression in cell transfer systems reported by others and seen in our earlier GVH experiments (24a, 25). In all of these cases, the transferred cells suppressed a host allotype different from that of the cell donor, presumably by inactivation of allotype-specific T helper cells or direct downregulation of the B cells themselves (27–29). In the current experiments, suppression of $IgG2a^b$ occurred after the double-parental chimeras received spleen cells from Igh^b bm12 mice (group III). By a hemagglutination assay a majority of the mice that were suppressed for $IgG2a^b$ also showed partial downregulation of $IgG2b^b$. IgM levels did not show any suppression, although overall IgM levels of the cognately recognized allotype were increased. We speculate that the suppression of the noncognate allotype may represent an attempt to downregulate the immune system in the setting of autoimmunity and may parallel the hyporesponsiveness to exogenous antigen challenge seen in the GVH model and spontaneous SLE (30–32). In any case, the fact that there was suppression does not affect the interpretation of a preference for direct T-B interactions for three reasons: (*a*) The recovery of the suppressed allotype of IgG2a in eight strongly suppressed mice did not result in antichromatin autoantibodies of that allotype; (*b*) the presence of the noncognate allotype in 5 mice that were only partially suppressed for IgG2a did not result in antichromatin autoantibodies of that allotype; (*c*) the IgM antichromatin antibodies were principally of the cognate allotype.

Our current work on autoimmunity parallels results with exogenous antigens (9, 10, 33–40). In an in vivo model cognate T-B collaboration was clearly preferred (38). In an in vitro system IgG isotype antibodies were dependent on cognate interactions, while IgM isotype could result from both cognate and bystander effects (41). In addition low concentrations of antigen favored cognate interactions, and this would be compatible with limited availability of intracellular autoantigens.

The results shown here are consistent with our hypothesis regarding the possible mechanisms of autoantibody production in SLE (42). We suggested that the generation of high titers of autoantibodies in SLE/GVH required direct recognition by T helper cells of B cell determinants, and specifically that T cells saw self non-MHC autoantigens complexed with an abnormally recognized Ia on the surface of B cells specific for such autoantigens. In the case of the alloreactive T helper cells in the present GVH chimera model, autoantigen would be recognized in conjunction with foreign Ia, although cognate T-B interactions could also occur if B cell foreign Ia were seen by itself (43). In human SLE an alteration in Ia may occur through mutations, viruses, or drugs, and then this would be recognized as foreign by self T cells. Cognate T-B interaction of these reactive T cells with altered Ia on autoreactive B cells would directly stimulate such cells to produce the spectrum of autoantibodies typical of SLE.

Summary

A chronic graft-versus-host reaction (GVH) induced in nonautoimmune mice causes a syndrome that closely resembles SLE. In this model, donor T cells react against incompatible host Ia structures and generate excessive help, which activates a subpopulation of self-reactive B cells. We have studied whether these self-reactive B cells are activated by direct interaction with alloreactive T cells or by nonspecific bystander effects. Two types of chimeras were made: double-parental chimeras, differing at both Ia and Igh allotype [B6.C20 + bm12 → (B6.C20 × bm12)F_1]; and control chimeras [(B6.C20 × bm12)F_1 → (B6.C20 × bm12)F_1]. A chronic GVH syndrome was induced in the chimeras by infusion of B6 or bm12 spleen cells. Coombs and antichromatin autoantibodies were measured using Igh allotype-specific immunoassays. The double-parental chimeras that received bm12 cells made autoantibodies principally of the Igh^a allotype, indicating that the bm12 T cells interacted only with the Ia^b-bearing host B cells. Conversely, double-parental chimeras that received B6 cells made mostly Igh^b autoantibodies, indicating direct cognate interaction with the Ia^{bm12}-bearing host B cells. The control chimeras made autoantibodies of both allotypes. These results indicate that autoantibodies in chronic GVH result from direct T-B interactions and not from nonspecific T cell-derived factors.

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