GENETIC REGULATION OF THE ANTIBODY RESPONSE TO *H-2D^b* ALLOANTIGENS IN MICE

III. Inhibition of the IgG Response to Noncongenic Cells by Preimmunization with Congenic Cells*

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Several genetic systems have been reported that regulate the antibody response to *H-2D^b* alloantigens in mice (1–5). Some of the described genes are linked to *H-2* (2, 3) whereas other genes are located outside the *H-2* complex (1, 4, 5).

A detailed investigation of the immunological phenomena that are regulated by one of the genetic systems located outside *H-2* has shown that some mice of the C57BL/10 series respond with only IgM alloantibodies to congenic immunizations with *H-2D^b* different cells (4–6). Most experiments have been performed with B10.A(5R) mice (5R)[1] immunized with C57BL/10 (B10) spleen cells. Even repeated injections with B10 cells yield only IgM antibodies. On the other hand, immunization with A.BY spleen cells, which carry other foreign cell surface antigens in addition to *H-2D^b*, leads to antibody production of the IgG type (4).

The experiments performed here were designed to investigate the effect of preimmunization with B10 cells on the response to subsequent immunization with A.BY cells. The results indicate that preimmunization with congenic cells can lead to inhibition of the normally occurring IgG response to noncongenic cells.

Materials and Methods

Mice and Immunizations. All animals used in the experiments reported here were from our own colonies of inbred mouse strains. B10.A(5R) mice (2–6-mo-old) were immunized with normal spleen cells of strains B10, A.BY, and A. The *H-2* haplotypes of these strains are shown in Table I. 5R belongs to the series of congenic strains of C57BL/10 origin (7); its *H-2* haplotype (*H-2^{i5}*) (8) is derived from a crossover between *H-2^a* and *H-2^b* occurring between *I-B* and *I-C* (9). B10 and A.BY mice both carry the *H-2D^b* haplotype, B10 on the same background as 5R, A.BY carrying the extra *H-2* genome of the unrelated strain A. Since 5R and B10 are congenic strains which differ at only part of the *H-2* complex, immunizations of 5R mice with B10 cells are directed solely against gene products governed by the *I-C^b*, *S^b*, or *D^b* regions of the *H-2* complex. Immunizations with A.BY cells are directed against the same products and, in addition, against non-*H-2* cell surface antigens, whereas immunizations with cells of strain A are directed against *H-2K^k*, *I-A^k*, and *I-B^k* plus cell surface antigens coded for by genes outside *H-2*.

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[1] *Abbreviations used in this paper:* B10, C57BL/10; 5R, B10.A(5R); 2-Me, 2-mercaptoethanol.

TABLE I
H-2 Haplotype of All Mouse Strains Used

	Haplotype	*H-2* Complex						Non-*H-2* background
		K	*I-A*	*I-B*	*I-C*	*S*	*D*	
Responding strain								
B10.A(5R)	*H-2*^{15}	*b*	*b*	*b*	*d*	*d*	*d*	C57BL/10
Immunizing cells								
B10	*H-2*^{b}	*b*	*b*	*b*	*b*	*b*	*b*	C57BL/10
A.BY	*H-2*^{b}	*b*	*b*	*b*	*b*	*b*	*b*	A
A	*H-2*^{a}	*k*	*k*	*k*	*d*	*d*	*d*	A
Target cells in cytotoxicity tests								
B10.HTG	*H-2*^{g}	*d*	*d*	*d*	*d*	*d*	*b*	C57BL/10
B10.BR	*H-2*^{k}	*k*	*k*	*k*	*k*	*k*	*k*	C57BL/10

Vertical bars indicate site of crossover.

5R mice received a first inoculation of 0.5×10^6 normal spleen cells injected subcutaneously; 2 wk later 5×10^6 cells were injected intraperitoneally, and the immunization was continued with biweekly intraperitoneal injections of 10×10^6 cells. In consecutive immunizations with cells from different strains, 5R mice were first injected with B10 or A.BY cells, and then at various intervals a new immunization was begun with either A.BY or A, starting again with 0.5×10^6 cells injected subcutaneously.

The immunized mice were bled from the retroorbital plexus 7 days after the fourth and fifth injection of the final immunization and were exsanguinated by heart puncture after the sixth immunization.

All antisera were stored at −70°C until tested. As target cells in the complement-mediated cytotoxicity test lymph node cells from strain B10.HTG were used to test for anti-*H-2D*^{b} antibodies and lymph node cells from B10.BR mice were chosen to test for antibodies against *H-2K*^{k} (Table I).

Complement-Mediated Cytotoxicity Test. The complement-mediated cytotoxicity test for antibody activity was performed on ^{51}Cr-labeled lymph node cells as previously described (4) with the following modification to allow for greater sensitivity and the use of 2-mercaptoethanol (2-Me) to test for 2-Me-sensitive and 2-Me-resistant antibodies. Instead of a one-step cytotoxicity assay with an incubation time of 1 h at room temperature, a two-step test was used in which the labeled target cells were incubated with antiserum in the cold, centrifuged, and then incubated at 37°C with guinea pig complement. Disulfide bond reduction of the *H-2* antibodies with 2-Me was carried out by incubating equal volumes of serum and 0.1 M 2-Me for 30 min at 37°C as described by Klein et al. (10), assuming that 2-Me-sensitive antibodies are of IgM type whereas 2-Me-resistant antibodies are of IgG type.

Results

We have previously described that B10.A(5R) mice produce only anti-*H-2D*^{b} antibodies of IgM type (2-Me-sensitive) when immunized with congenic B10 cells (4, 6). On the other hand they are capable of mounting an anti-*H-2D*^{b} IgG response if the antigen is presented on a cell that carries other foreign cell surface antigens in addition to *H-2D*^{b} as is the case with A.BY cells (Fig. 1).

We have now investigated the effect of preimmunization of 5R mice with B10 cells on the IgG response to a subsequent immunization with A.BY cells. 5R mice were injected once or twice with B10 cells or with A.BY cells as a control. Then a new immunization was started with 0.5×10^6 A.BY cells given subcuta-

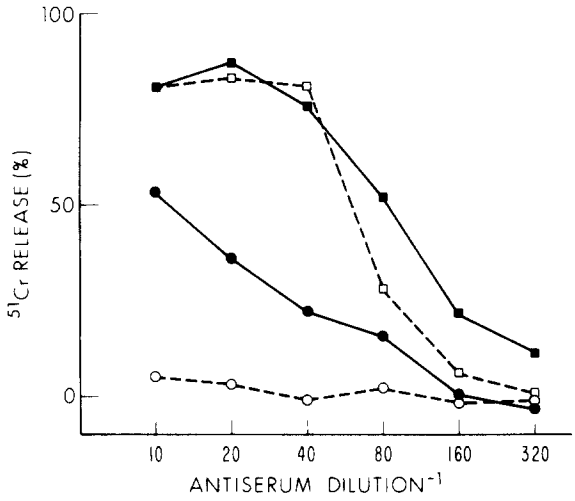

FIG. 1. Antibody levels as measured by a complement-mediated ^{51}Cr release cytotoxicity assay. Titration on B10.HTG target cells of sera collected 7 days after the sixth injection of 5R mice with A.BY (■—■, □- - -□) or B10 (●—●, ○- - -○) normal spleen cells. Straight lines: sera not treated with 2-Me. Dotted lines: sera after 2-Me treatment.

neously. The results are shown in Fig. 2. When the second immunization with A.BY cells was begun 7 days after one injection of B10 cells, the anti-A.BY response was of IgG type (panel A). No influence of the B10 injection on the anti-A.BY response could be seen. On the other hand if the subsequent immunization with A.BY cells was given after 5R mice had received two injections of B10 cells, the mice were unable to mount an IgG response to *H-2D*^{b} on A.BY; even after six injections with A.BY cells only IgM antibodies could be detected (panel C). Identical results were obtained if 5R mice were first injected with B10 cells three times.

Two controls were performed: Immunization with A.BY cells was begun 21 days after one injection of B10 cells to test whether the time after the first injection was critical (7 days after the second injection corresponds to 21 days after the first injection) or if the second injection with B10 cells was responsible for the failure to mount an IgG response to the immunization with A.BY cells. As shown in panel B of Fig. 2, the critical factor is not the time after the first injection but the second injection with B10 cells.

Panel D illustrates a control in which a second immunization with A.BY cells was started after two injections with A.BY cells. No effect on the IgG response is seen.

To test whether the inhibition of the IgG response was specific for *H-2D*^{b} and did not apply to another *H-2* antigen, 5R mice were injected twice with B10 cells and 7 days later an immunization with spleen cells of strain A was started. As can be seen in Fig. 3, 5R mice did produce IgG antibodies to *H-2K*^{k} when immunized with A cells indicating that failure to produce IgG antibodies to *H-2D*^{b} after previous injections with B10 cells is specific for the *H-2D*^{b} antigen carried on the congenic B10 cell.

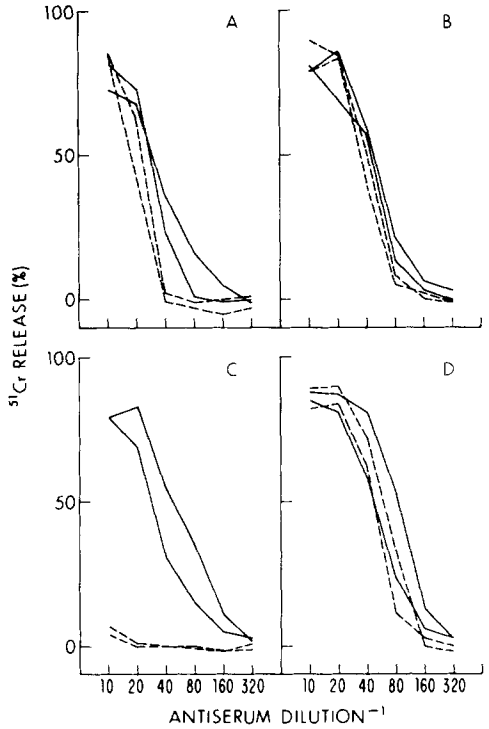

FIG. 2. Antibody levels as measured by a complement-mediated ^{51}Cr release cytotoxicity assay. Titration on B10.HTG target cells of sera collected 7 days after the fifth and sixth injection of 5R mice with A.BY normal spleen cells. Straight lines: sera not treated with 2-Me. Dotted lines: sera after 2-Me treatment. (A) 5R anti-B10 1-7 anti-A.BY: 7 days after a first injection with B10 cells an immunization with A.BY cells was begun. (B) 5R anti-B10 1-21 anti-A.BY: 21 days after a first injection with B10 cells an immunization with A.BY cells was begun. (C) 5R anti-B10 2-7 anti-A.BY: 7 days after the second injection with B10 cells an immunization with A.BY cells was begun. (D) 5R anti-A.BY 2-7 anti-A.BY: 7 days after the second injection with A.BY cells another immunization with A.BY cells was begun.

Discussion

In previous publications we have described that B10.A(5R) mice secrete only IgM type antibodies to *H-2D^b* if immunized with congenic B10 cells, whereas they are capable of mounting an IgG response to the same antigen if this antigen is presented on cells that carry additional foreign cell surface antigens, e.g. EL4 leukemia cells, or A.BY or BALB.B spleen cells (4, 6, 11).

The experiments reported here indicate that preimmunization of 5R mice with congenic B10 cells leads to failure to produce IgG antibodies in a subsequent immunization with A.BY cells, but does so only if 5R mice are injected with B10 cells twice. One injection of B10 cells does not have any effect. The lack of IgG response was shown to be specific for *H-2D^b* since it did not apply to a subsequent immunization against *H-2K^k*.

It has been described by Klein et al. (10) that one injection with *H-2* different cells leads to a thymus-independent alloantibody response of IgM type only.

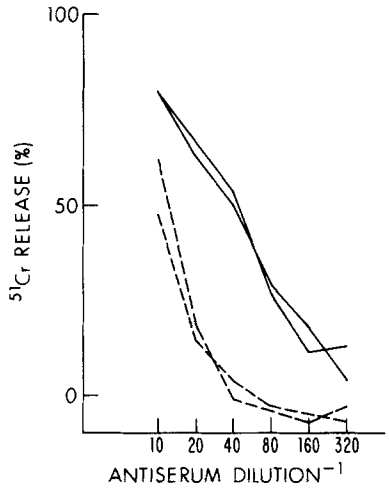

FIG. 3. Antibody levels as measured by a complement-mediated ^{51}Cr release cytotoxicity assay. 5R anti-B10 2-7 anti-A: 5R mice were first injected with B10 cells two times, 7 days after the second injection with B10 cells an immunization with normal spleen cells from strain A was begun. Titration on B10.BR target cells of sera collected 7 days after the fifth and sixth immunization. Straight lines: sera not treated with 2-Me. Dotted lines: sera after 2-Me treatment.

After a second injection a thymus-dependent IgG response can be detected that is abolished if the mice have been thymectomized, irradiated, and reconstituted with bone marrow cells (T-cell deficient "B" mice).

These results are in agreement with our findings that upon preimmunization with B10 cells one injection of congenic cells does not have any effect on the IgG response to A.BY cells, whereas after two injections of B10 cells the subsequent IgG response is abolished. It would seem that the first encounter with *H-2* different cells activates only IgM producing B cells and has no effect on thymus-derived lymphocytes; the second injection is necessary to involve the T cells that are responsible for the switch from IgM to IgG, and at this second injection, 5R mice display a defective response to B10 cells.

Applying Klein's data (which have been confirmed in part in our laboratory, D. Wernet, unpublished observation) to our experiments, it seems that B10.A(5R) mice behave like "B" mice in an *H-2D*^{b} congenic immunization, displaying a defect in T-cell function that renders them unable to mount a thymus-dependent IgG response under those circumstances. The fact that they can produce IgG antibodies if the same antigen is presented on a cell which carries additional foreign cell surface antigens has led to the following hypothesis (4): For 5R mice, *H-2D*^{b} alloantigens presented on a congenic background without additional foreign cell surface antigens are analogous to a "hapten" on a carrier that has no activating capacity for the helper T cells (a nonimmunogenic carrier). This "hapten" elicits a thymus-independent IgM response only, as has been demonstrated in other systems with autologous red blood cells as nonimmunogenic carriers (12, 13). For lack of activation of the helper T cells by the nonimmunogenic carrier the switch from the initial IgM antibody production to the secondary IgG response does not occur (14). Additional foreign cell surface

antigens convert the nonimmunogenic carrier into an immunogenic carrier that induces activation of T-cell help to elicit an IgG response. Helper activity by additional histocompatibility antigens in respect to another T-cell function (cell-mediated immunity) has been reported by DiMarco et al. (15).

The data reported here indicate that the failure to produce IgG antibodies may not be the result of simple lack of T-cell helper function but may involve tolerance or suppression at the T- or B-cell level. Lack of T-cell help for instance may lead to B-cell tolerance to the specific antigen so that the B-cells are unable to respond to the specific T-cell signal that is later provided by immunization with A.BY cells. B-cell tolerance has been described by Hamilton et al. (16) in a hapten carrier system with syngeneic erythrocytes.

At this moment we favor another possible model: Immunization of 5R mice with B10 cells elicits only on IgM response because of lack of carrier-dependent T-cell help. Instead of T-cell help, suppressor T cells are activated that act either directly on B cells (17), rendering them tolerant to subsequent helper T-cell signals, or suppressor T cells act on helper T cells (18) suppressing the IgG response that would otherwise be initiated. Suppression of the IgG response by suppressor T cells has been described in different systems (19, 20). This model implies different sets of T cells for helper function and suppressor function, a likely notion since Feldmann et al. have shown different Ly antigen phenotypes of helper and suppressor T cells induced in vitro (21).

With slight variation this model would also be consistent with the possibility that in normal immunized mice there are helper T cells and suppressor T cells present at the same time interacting with each other. The assumption would be that once the controlling factor of the helper T cell is lacking, the suppressor T cells become dominant over any T-cell help that might normally be induced by a second immunization with different cells.

At this moment there is no experimental evidence as to which model may be correct in the B10.A(5R) anti-B10 immunization. The lack of helper T-cell activation in *H-2*-congenic immunizations may be a fairly common phenomenon in inbred strains of mice (3) and may also apply to the antibody response to other cell surface antigens (22) and to tumor specific antigens on syngeneic tumor cells in which case activation of B-cell tolerance or T-cell-mediated suppression may strongly influence the outcome of tumor growth.

Summary

When B10.A(5R) mice ($H\text{-}2^{i5}$) are immunized with spleen cells from congenic B10 mice ($H\text{-}2^{b}$), they respond to alloantigens of the $H\text{-}2D^{b}$ region by producing antibodies of only IgM type. In contrast, they produce both IgM and IgG antibodies when immunized with A.BY cells ($H\text{-}2^{b}$) that carry other foreign cell surface antigens (non-*H-2*) in addition to $H\text{-}2D^{b}$. Preimmunization of 5R mice with two injections of congenic cells leads to an $H\text{-}2D^{b}$ specific inhibition of the IgG response to a subsequent immunization with A.BY cells. It is concluded that congenic B10 cells fail to activate helper T cells which are necessary to induce the switch from IgM to IgG production. Instead T- or B-cell tolerance may be induced with prohibits the subsequent IgG response to A.BY cells, possibly by way of suppressor T cells which may act either on B cells directly or via helper T cells.

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