

# GENETIC CONTROL OF THE IMMUNE RESPONSE TO COLLAGEN

## II. Antibody Responses Produced in Fetal Liver Restored Radiation Chimeras and Thymus Reconstituted F<sub>1</sub> Hybrid Nude Mice\*

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Immune responsiveness, as measured by the production of antibody to thymus-dependent antigens, has been shown in mice to be influenced by I region genes within the H-2 major histocompatibility complex. These genes appear to control two aspects of the induction of antibody synthesis: antigen-specific responsiveness (1-4) and the specificity of cellular recognition required for T-B-cell collaboration (5-7).

There now exists evidence that the antigenic and cellular recognition specificities exhibited by the population of mature T cells are characteristics acquired during maturation of the lymphoid system. Experiments using chimeric mice have shown that hematopoietic stem cells must differentiate in a high responder host environment to mature into lymphocytes that express a high immune response phenotype (8-9). In chimeric mice, there appears to be a selection for T cells that recognize host I region specificities and this restricts that population to recognition of a limited set of foreign antigens. The apparent relationship between the recognition of H-2 determinants and antigenic determinants remains a perplexing issue.

We have recently shown that the antibody response to soluble calf skin collagen is influenced by I region genes (10). In this report we examine the influence of the thymus on the expression of I region genes in the control of antibody synthesis by analyzing immune responses to collagen in two types of chimeric mice. First, we establish that H-2 determinants of the host affect the antigen responsiveness of the immune system. Lethally irradiated, high responder (C57BL/10) or low responder (B10.D2) mice, reconstituted with fetal liver cells from (C57BL/6 × BALB/c)F<sub>1</sub> hybrid mice, are shown to exhibit a responsiveness characteristic of the irradiated parental strains. Second, the involvement of the thymus in this selection process is demonstrated in a novel variation of the chimeric model reported by Kindred (11). Fetal thymus grafts from C57BL/6 or BALB/c mice were transplanted into congenitally athymic (nude) (C57BL/6 × BALB/c)F<sub>1</sub> hybrid mice. Antibody responses to

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collagen in thymus-grafted nude mice are characteristic of the strains from which the thymic grafts originated. These results indicate that the selection of cells on the basis of the recognition of a thymic element during T-cell maturation influences antigen responsiveness.

### Materials and Methods

*Mice.* BALB/cSt and C57BL/6St breeding pairs were purchased from the L. H. Strong Research Foundation (Del Mar, Calif.). C57BL/6 nu/nu breeding pairs were obtained from the Center for Cancer Research (Philadelphia, Pa.). BALB/c nu/nu mice were obtained from our own colony as previously described (12). C57BL/10J and B10.D2/nSn breeding pairs were purchased from The Jackson Laboratories (Bar Harbor, Maine). All parental and hybrid mice were bred in our colony.

*Antigens.* Sheep erythrocytes were purchased from Colorado Serum Co. (Denver, Colo.). Before injection, cells were washed three times in sterile Hanks' balanced salt solution (BSS)<sup>1</sup> and resuspended to a 10% solution (vol/vol). Calf skin collagen was purchased from Sigma Chemical Co. (St. Louis, Mo.) and prepared as described (10).

*Immunization.* Sheep erythrocytes were injected in 0.2 ml i.v. Mice were boosted 7 d later and bled 5 d after secondary injection. Collagen was emulsified in complete Freund's adjuvant and injected in 0.1 ml that was distributed between both hind foot pads. 3 wk later, an equivalent amount of collagen emulsified in incomplete Freund's adjuvant was injected subcutaneously above each hind foot. Mice were bled 2 wk after the secondary injection.

*Determination of Antibody Levels.* Antibody titers to sheep erythrocytes were determined by hemagglutination. IgM class antibodies were distinguished by sensitivity to preincubation with 0.1 M 2-mercaptoethanol (2-ME). Antibody activity which was resistant to 2-ME was considered to be IgG (13). The levels of serum antibody specific for collagen were determined by a radioactive antigen binding assayed as described (10). Data are presented as percentage <sup>125</sup>I-collagen bound versus the reciprocal of the serum dilution. Each mouse was individually bled and tested for collagen binding activity. The curves represent the mean of percent bound <sup>125</sup>I-collagen for each mouse in the experiment. Bars indicate the standard deviation from the mean. Data are calculated relative to a standard anti-collagen antibody in each assay.

*Radiation Chimeras.* Mice, 8–10 wk of age, were irradiated with 900 R at the 6 meV linear accelerator facility at the University of California, Irvine Medical Center. Mice were then reconstituted with 10<sup>7</sup> liver cells from 19-day-old fetuses. Mice were kept for 3 mo before use. Drinking water was supplemented with polymyxin B and neomycin sulfate (14) (Sigma Chemical Co.). Reconstituted mice showing any signs of infection were not used in this study.

*H-2 Typing.* Spleen cells were typed as H-2<sup>b</sup> or H-2<sup>d</sup> by sensitivity to antisera and complement. BALB/c anti-BALB.B (anti-H-2<sup>b</sup>) and BALB.B anti-BALB/c (anti-H-2<sup>d</sup>) were prepared by weekly injections of 2 × 10<sup>7</sup> cells/mouse. To determine sensitivity to antisera lysis, 5 × 10<sup>6</sup> spleen cells were incubated in 50 μl of a 1:5 dilution of antisera on ice for 15 min. This suspension was then incubated at 37°C for 30 min in the presence of 50 μl of 1:5 dilution of guinea pig complement absorbed with agarose. The percent of viable nucleated cells were scored by fluorescent staining in the presence of 1 μg/ml fluorescein diacetate. Using this procedure, viability after treatment with the nonreactive antisera on parental B10 and B10.D2 mice was 95% of control and with the reactive antisera was <5%. True chimerism was assumed in F<sub>1</sub> → parental mice if spleen cells were 95% sensitive to both antisera.

*Construction of Chimeras from Nude Mice.* (C57BL/6 × BALB/c)F<sub>1</sub> hybrid homozygous nude mice were obtained by breeding C57BL/6.nu/+ × BALB/c.nu/+ heterozygous mice. Thymus donors were 15–19-d fetal C57BL/6St or BALB/c mice.

Fetal thymuses were aseptically removed and bathed in BSS on ice until implantation. (BALB/c × C57BL/6)F<sub>1</sub> nu/nu mice between 4 and 8 wk of age were anesthetized with nembutal. Two thymus equivalents (four lobes) were placed under the left kidney capsule of each mouse. Grafted animals were allowed to mature for at least 12 wk before immunization.

<sup>1</sup> Abbreviations used in this paper: BSS, Hanks' balanced salt solution; 2-ME, 2-mercaptoethanol; SRBC, sheep erythrocytes.

By this time, parental strain lymphocytes in the thymus and periphery have been shown to be undetectable (15).

### Results and Discussion

The levels of antibody synthesized in response to immunization with collagen are controlled by multiple H-2 linked genes (10). To test whether this control occurs during maturation and differentiation of the immune system, high responder, C57BL/10J (H-2<sup>b</sup>), and low responder, B10.D2/nSn (H-2<sup>d</sup>) mice were lethally irradiated and reconstituted with (BALB/c × C57BL/6)F<sub>1</sub> (H-2<sup>d/b</sup>) fetal liver cells. If the response phenotype is inherited and determined by the genotype of the lymphoid system, the irradiated background cells of a host animal should not affect immune responsiveness. However, if immune responsiveness is determined as a result of a selection for T cells which can recognize self-histocompatibility determinants, then the irradiated background would be predicted to determine the antibody response phenotype. Because there is no difference in antibody titers elicited in H-2<sup>b</sup> and H-2<sup>d</sup> mice by immunization with sheep erythrocytes (SRBC), this antibody response was used as an indication of immune reconstitution and competence. Table I presents a summary of data from fetal liver restored radiation chimeras. Fetal liver-restored B10 and B10.D2 mice immunized with SRBC produced similar titers of 2-ME-resistant anti-SRBC antibodies (Table I). To examine the effect that H-2 determinants may have on the maturation of immunocompetent cells, these chimeras were then tested for the ability to synthesize antibody in response to immunizations with collagen. For comparison, responsiveness was tested in control B10 and B10.D2 mice (Fig. 1). The results of this experiment show that in response to immunizations with collagen, F<sub>1</sub> → B10 chimeric mice produce significantly higher levels of antibody than F<sub>1</sub> → B10.D2 chimeric mice. Because H-2<sup>b</sup> (B10) mice are high responders to collagen and H-2<sup>d</sup> (B10.D2) mice are low responders, these results indicate that the expression of H-2 determinants in the irradiated parental host causes the F<sub>1</sub> cells that reconstitute the immune system to exhibit an immune responsiveness characteristic of the host.

The involvement of the thymus in the determination of immune responsiveness to collagen was tested in chimeric mice in the following way. (BALB/c × C57BL/6)F<sub>1</sub> nude mice were grafted with thymuses from 15- to 19-d fetal mice of either C57BL/6 (H-2<sup>b</sup>) or BALB/c (H-2<sup>d</sup>) parental strains. Ungrafted F<sub>1</sub> nude mice are completely unresponsive to stimulation by the T-cell mitogen concanavalin A and synthesize no detectable 2-ME resistant antibody in response to multiple injections with SRBC (data not shown). As such, significant titers of 2-ME-resistant antibody in thymus-grafted nude mice are considered indicative of T-cell reconstitution. 12 wk after grafting, chimeric mice were immunized with SRBC as described in Methods. The anti-SRBC titers of the grafted or ungrafted F<sub>1</sub> nude mice presented in Table II reveal that there exists no consistent difference in 2-ME-resistant antibody titers elicited by SRBC in C57BL/6 and BALB/c thymic transplanted chimeras. These mice are considered equally immunocompetent for a thymus-dependent antibody response. Fig. 2 presents the antibody levels elicited by subsequent immunizations with collagen in these F<sub>1</sub> nude mice grafted with a high (H-2<sup>b</sup>) or low (H-2<sup>d</sup>) responder parental strain thymus tissue. For comparison, responses are also shown for C57BL/6 and BALB/c strain mice. The levels of antibody produced to collagen in C57BL/6 (thymus) → F<sub>1</sub> (nude) chimeras are significantly higher than the levels produced by

TABLE I  
Antibody Response to SRBC in Radiation Chimeras\*

Fetal liver source‡	Irradiated host	Hemagglutination titer§	
		-2-ME	+2-ME
1. (C57BL/6St × BALB/cSt)F <sub>1</sub>	C57BL/10J	1/160	1/80
2. (C57BL/6St × BALB/cSt)F <sub>1</sub>	C57BL/10J	1/80	1/40
3. (C57BL/6St × BALB/cSt)F <sub>1</sub>	C57BL/10J	1/80	1/40
4. (C57BL/6St × BALB/cSt)F <sub>1</sub>	B10.D2/nSn	1/80	1/40
5. (C57BL/6St × BALB/cSt)F <sub>1</sub>	B10.D2/nSn	1/80	1/40

\* Mice were considered to be true chimeras if spleen cells showed no significant difference in susceptibility to lysis with anti-H-2<sup>b</sup> and anti-H-2<sup>d</sup> antisera from control (C57BL/6 × BALB/c)F<sub>1</sub> mice.

‡ Fetal livers were taken from 19-d fetal mice.

§ Mice were injected with 0.2 ml 10% SRBC, boosted 7 d later, and bled 5 d after the secondary immunization.

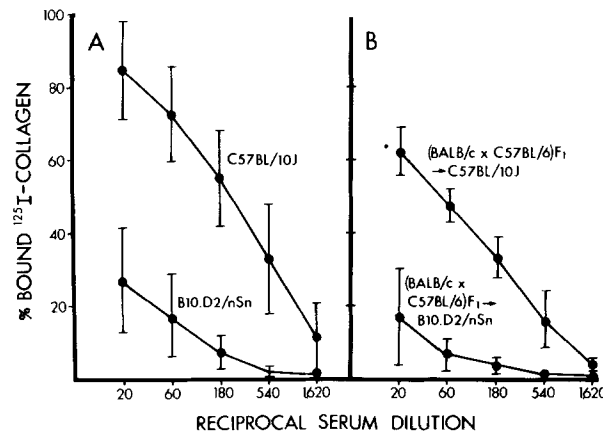


FIG. 1. Antibody response to collagen in radiation chimeras. Mice were injected with 50  $\mu$ g of collagen emulsified in complete Freund's adjuvant. 3 wk later mice were boosted and bled 2 wk after the secondary immunization. Dilution curves represent the mean of the percentage of bound <sup>125</sup>I-collagen for each animal listed in Table I.

BALB/c (thymus) → F<sub>1</sub> (nude) chimeras. These results indicate that between animals which differ by only the origin of the thymus, immune responsiveness is significantly different. BALB/c thymus-grafted F<sub>1</sub> nude mice produce levels of anti-collagen antibody that are characteristic of BALB/c mice and C57BL/6 thymus-grafted F<sub>1</sub> nude mice produce levels of anti-collagen antibody that are characteristic of C57BL/6 mice. In agreement, Zinkernagel et al. have shown that F<sub>1</sub> nude mice, grafted with a parental strain thymus, are restricted to killing reactions against virus-infected cells bearing the same H-2 haplotype as the thymus (16).

The results of this study show that the expression of H-2 genes in the tissue of the thymus are involved in determining the level of antibody responses in mice. Other studies have shown that T cells exhibit two recognition functions, and I region control of antibody production has been linked to the expression of these two T-cell recognition processes. First, during maturation, helper T cells have been shown to acquire the ability to recognize I region-encoded antigenic specificities, whereas cytotoxic T

TABLE II  
 Antibody Response to SRBC in (BALB/c × C57BL/6)F<sub>1</sub> (nu/nu) Nude Mice

Genotype of host	Transplanted thymus source	Hemagglutination titer*	
		-2-ME	+2-ME
1. (BALB/c × C57BL/6)F <sub>1</sub> nu/nu	C57BL/6	1/160	1/40
2. (BALB/c × C57BL/6)F <sub>1</sub> nu/nu	C57BL/6	1/1,280	1/320
3. (BALB/c × C57BL/6)F <sub>1</sub> nu/nu	C57BL/6	1/320	1/160
4. (BALB/c × C57BL/6)F <sub>1</sub> nu/nu	C57BL/6	1/320	1/320
5. (BALB/c × C57BL/6)F <sub>1</sub> nu/nu	BALB/c	1/160	1/80
6. (BALB/c × C57BL/6)F <sub>1</sub> nu/nu	BALB/c	1/640	1/320
7. (BALB/c × C57BL/6)F <sub>1</sub> nu/nu	BALB/c	1/640	1/320
8. (BALB/c × C57BL/6)F <sub>1</sub> nu/nu	—	1/20	<1/10
9. (BALB/c × C57BL/6)F <sub>1</sub> nu/nu	—	1/20	<1/10
10. (BALB/c × C57BL/6)F <sub>1</sub> nu/+	—	1/640	1/320
11. (BALB/c × C57BL/6)F <sub>1</sub> nu/+	—	1/640	1/320

\* See Table I.

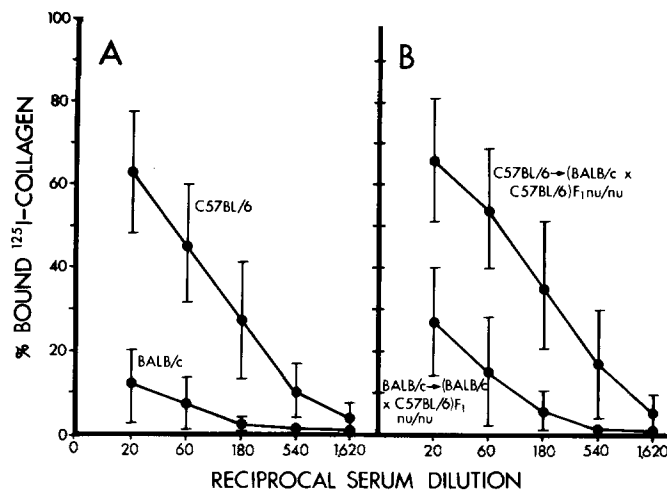


FIG. 2. Antibody response to collagen in thymus-transplanted nude chimeras. Dilution curves represent the mean of the percent bound <sup>125</sup>I-collagen for each animal listed in Table II. See Fig. 1 for protocol.

cells learn to recognize K and D region-encoded antigenic specificities (8, 9, 17-20). This process is considered to occur in thymic tissue (21). Second, immune responsiveness exhibited to a particular antigen appears to be linked to the H-2-encoded specificities that T cells have learned to recognize. The suggestion has been made that the genetic information required to synthesize receptors on T cells for both H-2 and foreign determinants is contained in germ-line genes that code for recognition structures directed against the major histocompatibility antigens of the species. The selection of genes to form a receptor on T cells that binds H-2 determinants limits the subsequent diversity of antigen recognition by constraining the genetic information available for the synthesis of antigen receptors (9, 21). An alternative suggestion is that T cells are selected to recognize an H-2 determinant present on a molecule which must associate with antigens presented in an immunogenic form. Low responsiveness

is the result of a low affinity association between I region-encoded molecules and antigen (22). Whereas such interpretations are difficult to rationalize, it appears that the products of the I region genes which control levels of antibody responses in mice are identical to I region-encoded recognition determinants expressed both in the thymus and on effector cell types.

### Summary

The level of antibody produced in response to calf skin collagen in mice is influenced by genes which are closely linked to the I region of the H-2 major histocompatibility complex. This influence is shown to be expressed during lymphoid maturation by testing the antibody responsiveness to collagen in two types of chimeric mice. First, high responder and low responder parental strain mice were lethally irradiated and restored with fetal liver cells from (high  $\times$  low responder) F<sub>1</sub> mice. These F<sub>1</sub>  $\rightarrow$  parent chimeras exhibited an immune response phenotype characteristic of the irradiated parental strain animals, establishing that H-2 determinants of the host affect antigen responsiveness. Second, (high  $\times$  low responder) F<sub>1</sub> congenitally athymic (nude) mice were restored with fetal thymus transplants from either high or low responder parental strain mice. After a period of maturation these mice were shown to be competent for a T-dependent IgG response to SRBC. The responsiveness to collagen in these mice was characteristic of the parental strain thymus donors, indicating that the expression of H-2 determinants in thymic tissue during lymphoid maturation influences the antibody response phenotype expressed by mice.

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