

REGULATION OF IgE ANTIBODY PRODUCTION BY SERUM  
MOLECULES

III. Induction of Suppressive Activity by  
Allogeneic Lymphoid Cell Interactions and  
Suppression of IgE Synthesis by the Allogeneic Effect\*

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Antibody responses of the IgE class are, like other immunoglobulin classes, regulated by a finely-tuned network of complex cellular and molecular interactions (1). Previous studies conducted in our laboratory (2, 3) have provided new insights into the differences in control mechanisms that result in individuals manifesting either the high (allergic) or low (nonallergic) IgE responder phenotype. These experiments have shown that certain manipulations (i.e. low dose X-irradiation) convert normally low responder mice to high IgE responders, apparently by diminishing a suppressor T-cell mechanism which normally dampens, rather selectively, IgE antibody production in such individuals. Similar findings have been made by Watanabe et al. (4).

Recently, we have been studying the types of manipulations that could reverse the high IgE responsive state back to a low one. These studies (2, 3, 5, 6) have demonstrated that the high IgE responses induced in low responder mice can be substantially diminished, and even abolished, by passively transfusing serum or ascitic fluid from donor mice previously inoculated with mycobacterial-containing complete Freund's adjuvant (CFA). Because the suppressive activity of CFA-immune serum or ascitic fluid is so highly selective for IgE antibody production, we have recently termed these serum substances suppressive factors of allergy (SFA) (2, 3).

The present study was undertaken to determine whether alternative means, particularly those that avoid administration of CFA, could be devised for the induction of SFA. Herein, we report the effectiveness of allogeneic lymphoid cell interactions in inducing SFA, both in vivo and in vitro, as well as the potent suppressive effects of an in vivo allogeneic effect on irradiation enhanced IgE antibody production in low responder mice.

**Materials and Methods**

The proteins, reagents, and preparation of hapten-protein conjugates, the inbred mice and immunization methods, the rats employed for measurement of IgE antibodies by passive cutaneous anaphylaxis (PCA), measurements of serum IgE and IgG 2,4 dinitrophenyl

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(DNP)-specific antibodies, the regimen for administration of donor serum (an identical regimen was employed for administration of culture supernatants) to test mice, and the X-irradiation procedures were the same as those described previously (5, 6). The dose of irradiation employed was 250 R in most instances, except in one experiment in which it was increased to 350 R.

Large-scale syngeneic and allogeneic mixed lymphocyte cultures (MLC) were set up as previously described (7). 14 ml of cell suspensions containing  $1.25 \times 10^6$  SJL responder cells and  $2.5 \times 10^6$  2,500 R irradiated target cells per ml were added to each Falcon 3013 tissue culture flask. (BioQuest, BBL, & Falcon Products, Becton, Dickinson & Co., Cockeysville, Md.) Target cells were irradiated SJL cells in syngeneic MLC, and a mixture of equal numbers of BALB/c, DBA/2, C57BL/6, C3H, and SWR cells in allogeneic MLC. After 4 d, small portions of each flask were transferred to microtiter plate wells, pulsed for 18 h with 1  $\mu$ Ci tritiated thymidine, and then harvested. Such analyses demonstrated 4,000 cpm in syngeneic versus  $\approx 170,000$  cpm in the allogeneic MLC, i.e. a stimulation index of  $\approx 40$ -fold. After 5 d of incubation, supernates from the large flask cultures were collected by centrifugation, concentrated 25-fold (PM-10 membranes, Amicon Corp., Lexington, Mass.), and stored frozen at  $-70^\circ\text{C}$  until used.

### Results and Discussion

Fig. 1 illustrates that irradiation-enhanced primary IgE responses (upper panel) of SJL mice can be totally suppressed by passive transfusion of appropriate quantities of allogeneic C57BL/6 lymphocytes; syngeneic SJL spleen cells induced moderate suppression. When a second transfusion preceded secondary challenge on day 18, a similar suppressive effect of allogeneic lymphocytes was evident, while syngeneic cells had absolutely no effect (bottom panel). The suppressive effect of transferred allogeneic lymphocytes was restricted to the IgE class (e.g. IgG responses were 9.2, 8.7, 10.4, and 10.1  $\mu\text{g}/\text{ml}$  on day 14 and 54.3, 48.7, 58.6, and 60.4  $\mu\text{g}/\text{ml}$  on day 25 for groups I-IV, respectively).

We next sought to determine whether the allogeneic effect might induce production of SFA, or SFA-like material, in the serum of low responder mice. In Fig. 2, note the remarkable 64-fold enhancement of responses in irradiated group II mice. Passive transfusion of normal SJL serum only partially suppressed, whereas serum from SJL mice which had received allogeneic BALB/c cells completely suppressed these enhanced IgE responses. Again, there were no significant differences in the magnitudes of IgG anti-DNP antibody responses made by these various groups.

The experiment summarized in Fig. 3 was designed to determine whether similar suppressive activities are elaborated into the supernates of MLC. Once again, low dose X-irradiation enhanced IgE antibody production by 64-fold. Administration of either syngeneic or allogeneic MLC supernates resulted in a clear depression of such enhanced responses in a dose-related, and interestingly biphasic, pattern. Thus, in doses of 0.2 or 0.3 ml/injection, significant suppression occurred with either type of supernate although suppression was significantly greater with 0.3 ml of allogeneic than with syngeneic supernate. In both cases, however, administration of 0.4 ml suppressed significantly less than the 0.3 ml dose.

These experiments demonstrate that induction of an *in vivo* allogeneic effect is an effective means for exerting potent suppressive influences on IgE antibody production in low responder mice which have been converted to high responder status by low dose X-irradiation. Furthermore, this is clearly a successful method for stimulating production of IgE-selective suppressive molecules, SFA, that circulate in the serum of low IgE responder mice. Indeed, our available evidence suggests that such allogeneic effect serum is more potent in suppressive activity than serum obtained from a CFA-immune donor mouse. It should be emphasized that it would be premature to

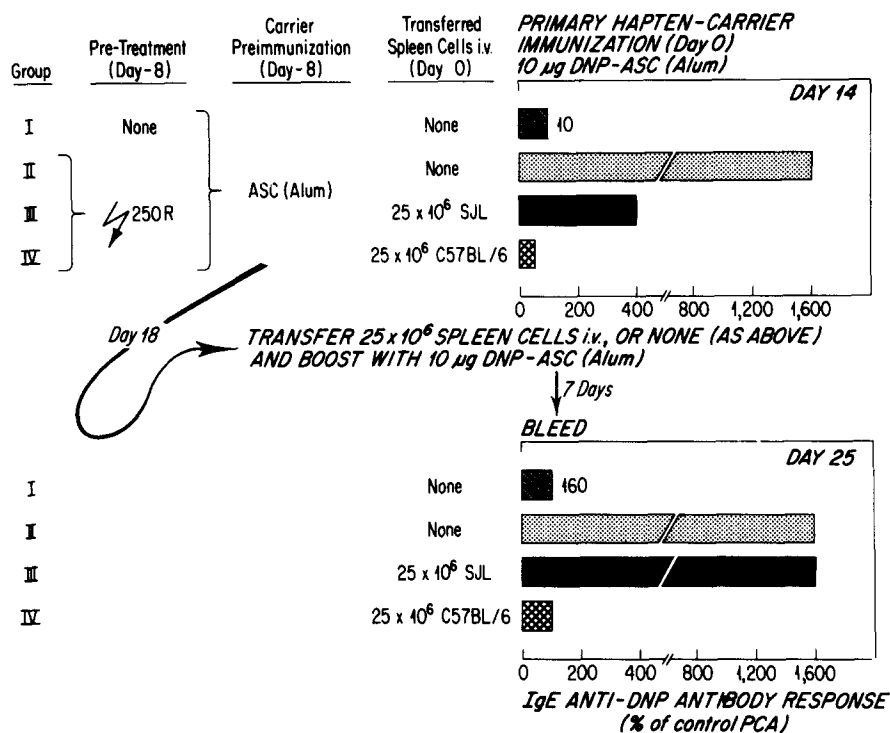


FIG. 1. Suppression of irradiation-enhanced IgE antibody production in low responder SJL mice by passively transferred allogeneic cells. SJL mice were either not pretreated or pretreated with 250 R X-irradiation shortly before preimmunization with 10 µg of *Ascaris suum* (ASC) in alum on day -8. On day 0 all mice were primarily immunized with 10 µg of DNP-ASC in alum and then given either no cells, 25 × 10<sup>6</sup> syngeneic (SJL), or 25 × 10<sup>6</sup> allogeneic (C57BL/6) cells intravenously. On day 18, the groups of mice were again transfused with cells, or not, (as on day 0) and then secondarily boosted with 10 µg of DNP-ASC in alum. The IgE antibody responses on day 14 (top panel) and day 25 (bottom panel) are presented as percent of control responses in group I, with the corresponding control PCA value listed beside the data bar. Although not illustrated, IgG antibody responses did not differ significantly on either day 14 or day 25 among the various groups of mice.

conclude that the mechanism of suppression by allogeneic cell transfusions is related solely to the production of circulating SFA. Moreover, further work is required to determine whether the suppressive activity found in the serum of recipients of allogeneic cells is the same as that induced by inoculation of mycobacterial-containing CFA. Further investigation should also clarify the relationship(s) of SFA detected in our system to similar IgE-selective soluble factors either extracted from normal low responder spleen cells (9) or elaborated in culture after exposure of primed spleen cells to the priming antigen (10).

The rationale for employing allogeneic lymphocytes for this particular purpose stems from earlier work which demonstrated that histoincompatible cells provide potent regulatory effects on a variety of immune responses (1). In earlier studies, we found that allogeneic lymphocytes failed to exert helper activities and more often exerted suppressor effects on the IgE response (8). This may be partly explained by the present results which demonstrate that one important consequence of allogeneic cell interactions in vivo is the production of large quantities of SFA, or SFA-like materials.

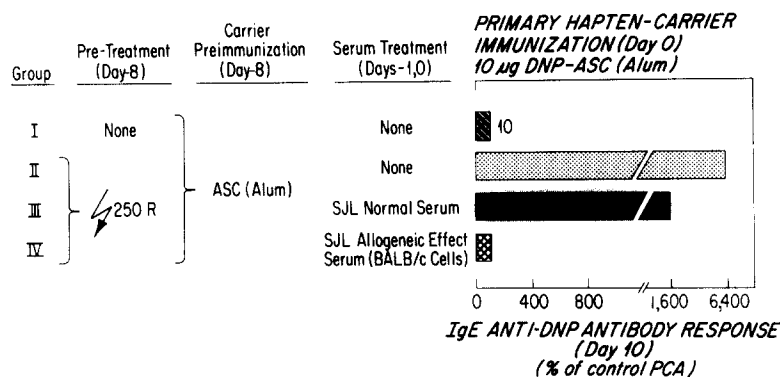


FIG. 2. Suppression of irradiation-enhanced primary IgE antibody production in SJL mice by passive transfer of allogeneic effect serum. The protocol summarized on the left of the figure was employed. Allogeneic effect serum was obtained from normal SJL mice which were injected intravenously with  $25 \times 10^6$  spleen cells from normal BALB/c donors and then bled 6 and 7 d later. Passive serum transfusions were administered as previously described (5, 6). The IgE anti-DNP antibody responses on day 10 after immunization with DNP-ASC are illustrated as percent of control with the group I control values listed beside the corresponding bar. There were no significant differences in levels of IgG anti-DNP antibody among the different groups.

Also shown in this report is the capacity to obtain suppressive activity from in vitro MLC supernates although the identity or similarity (or not) of such molecules to those in serum of either CFA-primed or allogeneic cell-transfused donors must await future biochemical analysis. Furthermore, the cellular source of the biologically-active supernatant molecules, i.e. whether from responder and/or target cells, has yet to be determined. The fact that suppressive material could be detected in the supernate of syngeneic MLC is not surprising because we have previously documented that SFA is produced in vivo, although in low quantities, without the need for any overt manipulation. It should be noted that the results are not explained by the presence of fetal calf serum (FCS) components in these cultures because inoculation of comparable quantities of FCS failed to mimic the effects observed with the MLC supernates.

The interesting biphasic pattern of effects observed with the MLC supernates cannot be addressed in detail here. It must suffice to point out that we have recently discovered the existence of opposing enhancing activity in the serum of both low responder and high responder mice. This enhancing activity belongs to a distinct molecular species from SFA, is similarly selective in activity for IgE responses and, interestingly, is stimulated by similar manipulations that induce production of SFA. These studies, which will be reported shortly,<sup>1</sup> indicate that the IgE response phenotype of a given individual at a given time most likely reflects the net balance of these two opposing biological activities that are produced during physiological responses of the IgE class.

The results presented here therefore illustrate an alternative means for potentially manipulating IgE antibody production in a selective fashion by using an approach that avoids the need for administering mycobacterial-containing materials, which

<sup>1</sup> Katz, D. H., R. F. Bargatze, C. A. Bogowitz, and L. R. Katz. 1978. Regulation of IgE antibody production by serum molecules. IV. Complete Freund's adjuvant induces both enhancing and suppressive activities detectable in the serum of low and high responder mice. Manuscript submitted for publication.

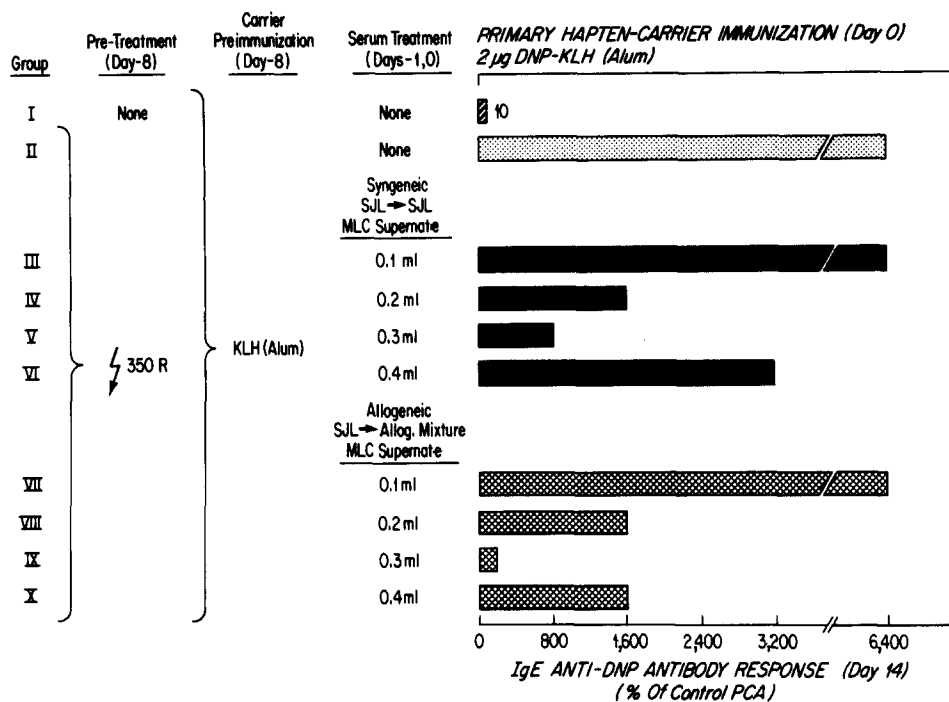


FIG. 3. Suppression of irradiation-enhanced primary IgE responses in low responder SJL mice by passive transfer of mixed lymphocyte culture supernates. The protocol summarized on the left of the figure was employed. Supernatant fluid from either syngeneic or allogeneic MLC were injected as previously described (5, 6). The quantities listed beside each group denote the amounts of supernatant fluid administered per injection. The IgE antibody responses on day 14 after immunization with DNP-keyhole limpet hemocyanin (KLH) are illustrated as percent of control with the group I control value listed beside the corresponding bar.

may be undesirable in certain circumstances and, moreover, suggest an approach for producing a selective IgE suppressive material in a completely *in vitro* system.

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