INDUCTION OF TOLERANCE TO
1-FLUORO-2,4-DINITROBENZENE CONTACT SENSITIVITY
WITH HAPTEN-MODIFIED LYMPHOID CELLS
II. Selective Tolerance in F₁ Mice of T Cell Subsets
Recognizing 1-fluoro-2,4-dinitrobenzene Associated with
Parental Major Histocompatibility Complex Antigens*

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Recent investigations in cellular immunology have shown that activation of thymus-derived lymphocytes (T cells) for cytotoxicity (1, 2), helper activity in antibody production (3-5), and in the adoptive transfer of delayed-type hypersensitivity (6, 7) requires at least the recognition of antigen in association with major histocompatibility complex (MHC) gene products. We have reported (8) that in contact sensitivity to 1-fluoro-2,4-dinitrobenzene (DNFB), the rapid induction of T cell tolerance by intravenous injection of in vitro haptenated cells requires Ia-bearing cells compatible with the recipient. Because it is independent of any demonstrable suppressor cells (9, 10), we believe this rapidly induced tolerant state is a result of the inhibition of antigen-reactive T cell clones. Recent experiments indicate that within an F₁ (i.e., A × B) animal, there exist separate clones of antigen-reactive T cells. One set of cells recognizes antigen in the context of one of the parental MHC, e.g., Ag + MHC – A, and another set recognizes Ag + MHC – B (4, 5, 8, 11). If our hypothesis that rapidly induced tolerance involves inhibition of antigen-reactive T cells is correct, then it should be possible to tolerize each clone separately. This report presents evidence that in contact sensitivity to DNFB one can tolerize (A × B)F₁ mice so that cells recognizing DNP + MHC – A are unresponsive, whereas cells recognizing DNP + MHC – B are fully reactive, and vice versa.

Materials and Methods

Mice. 2- to 4-mo-old animals were used throughout these investigations. BALB/c females were obtained from Cumberland View Farms, Clinton, Tenn.; CBA/J males from The Jackson Laboratories, Bar Harbor, Maine; and (BALB/c × CBA/J)F₁ of both sexes were raised at the University of Colorado Medical Center (Denver, Colo.). Mice were age matched for each experiment.

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Antigen. DNFB was obtained from Sigma Chemical Co., St. Louis, Mo.

Preparation of Hapten-modified Spleen Cells. Erythrocyte-free spleen suspensions were prepared in Hank's balanced salt solution (HBSS) and were dinitrophenylated as previously described (11). After haptenation, the DNFB-modified spleen cells (DNP-SC) were washed twice in HBSS and resuspended to the desired cell concentration for injection.

Cyclophosphamide Treatment. To eliminate the precursors of suppressor T cells (Ts), mice were injected intravenously 2 d before tolerization with 150 mg/kg body wt of cyclophosphamide (Cy) (Cytoxan, Mead Johnson & Co., Evansville, Ind.) diluted in sterile water.

Induction of Tolerance. F1 animals were injected intravenously with either sham- or DNP-SC from parental (BALB/c or CBA/J) or (BALB/c × CBA/J)F1 donors 1 d before sensitization.

Sensitization and Elicitation. Animals (four per group) were sensitized on the shaved abdominal skin with 25 µl of 0.5% DNFB on days 0 and 1. To determine the extent of donor tolerance, members of each F1 donor group were ear challenged with 0.2% DNFB on day 5, and the 24-h ear swelling was quantitated with a Mitutoya Engineer's micrometer. The increment of ear swelling is expressed as the mean in units of 10⁻⁴ in. ± SEM. The amount of donor tolerance obtained was calculated by comparing the response of each experimental group with that of positive (sensitized and ear challenged) and negative (ear challenged only) control groups.

Transfer of Sensitivity. For transfer of sensitivity, draining lymph nodes from F1 donor animals were collected on day 4, 2 d after the last painting. Single cell suspensions were prepared in balanced salt solution and 50 X 10⁶ donor lymphocytes were injected intravenously into naive BALB/c or CBA/J recipients. 1 h later, the recipients were challenged on the ears with 0.2% DNFB, and the 24-h swelling was quantitated as described above.

Statistical Analysis. The statistical significance of the differences between the experimental and control groups was determined by the Student's t test. The experiments reported here were done at least four times, and representative results are presented.

Results and Discussion

Unresponsiveness to DNFB contact sensitivity occurs after injection of hapten-modified cells (9, 10, 12) and is mediated by at least two distinguishable mechanisms: donor tolerance and the generation of Ts (10). Donor tolerance is antigen specific, long lasting, nontransferable, and insensitive to regimens known to inactivate Ts precursors (i.e., Cy) (9, 10, 12-14). This form of tolerance is most efficiently induced by haptenated, Ia-bearing cells (8, 15). Thus, we proposed that DNP plus Ia antigens inactivate the T cell clones required for expression of contact sensitivity.

To test this hypothesis, we asked whether it was possible to tolerize specifically one of the two T cell subpopulations within an (A × B)F1 host (4, 5, 7, 11) (e.g., that recognizing DNFB on the background of parent A) while not affecting the development of immunity by the remaining T cell subset (that recognizing DNFB on the background of parent B). Tolerance was produced in F1 donors by first pretreating with Cy on day -3, followed on day -1 by injection of either sham- or DNP-SC from parent or F1 strains. On days 0 and 1, the mice were sensitized epicutaneously with DNFB. F1 lymph node (LN) cells were removed on day 4 and adoptively transferred to naive recipients of both parental strains. The recipients were then ear challenged 1 h later, and swelling was measured 24 h later. The percentage of the maximum response transferred was calculated by comparing the response of each recipient experimental group with that of recipients receiving lymphocytes from sham-pretreated F1 donors.

To assess the extent of tolerance in the F1 donor animals, F1 mice injected with sham- or DNP-SC were challenged on day 5. Similar levels of unresponsiveness were produced in these animals regardless of the source of the DNP-SC. Thus, the mean tolerance in F1 donors pretreated with F1 DNP-SC was 50% (range: 42-66%), in F1
Table I
Tolerization of Parental Strain-Reactive Cells in F1 Mice

| Group | Pretreatment of F1 donor* | Recipient of F1 LN cells† | Ear swelling in × 10^-4 ± SEM§ | Percentage of maximum response transferred % |
|-------|--------------------------|-------------------------|-------------------------------|-------------------------------------------|
| A     | Sham-F1 cells            | BALB/c                  | 25.5 ± 3.3                    | 100                                       |
| B     | DNP-F1 cells             | BALB/c                  | 13.9 ± 1.9                    | 40†                                      |
| C     | —                        | BALB/c                  | 6.0 ± 1.4                     | 0                                        |
| D     | Sham-F1 cells            | CBA/J                   | 37.7 ± 3.6                    | 100                                      |
| E     | DNP-F1 cells             | CBA/J                   | 17.8 ± 1.7                    | 35¶                                      |
| F     | —                        | CBA/J                   | 7.0 ± 1.2                     | 0                                        |

* 2 d after Cy pretreatment, animals were injected intravenously with 40 × 10^6 F1 spleen cells that were either sham or DNP modified. Sensitization occurred the following 2 d.
† On day 4, draining LN were removed from F1 donor animals, and 50 × 10^6 cells were intravenously injected into naive parental strain recipients. The recipients were then ear challenged 1 h later.
§ The values represent the mean 24-h ear swelling (five animals per group) ± SEM.
¶ The percentage of the maximum response transferred was calculated by comparing the response of each recipient experimental group to that of sham (recipients receiving LN cells from sham-pretreated F1 donors) and negative (recipients receiving ear challenge only) control groups: percentage of maximum response transferred = (experimental-negative/sham-negative) × 100%.

When F1 donor mice are tolerized with F1 DNP-SC and then sensitized, the ability of LN cells to transfer sensitivity to parental strain recipients is significantly reduced (Table I). The top half of the table shows results with BALB/c recipients. LN cells from F1 donors tolerized with F1 DNP-SC transferred only 40% of the response transferred by LN cells from sham-pretreated donors (compare groups B and A; P < 0.001). Similar results were obtained with CBA/J recipients (bottom half of Table I). In this case, only 35% of the maximum response was transferred by LN cells from F1 donors tolerized with F1 DNP-SC (compare groups E and D; P < 0.001). These results indicate that pretreatment of F1 mice with F1 DNP-SC tolerizes T cells responsible for the transfer of contact sensitivity to both parental strain recipients.

We next investigated whether pretreating F1 donor animals with DNP-SC from one parent strain would selectively tolerate those T cells recognizing DNP in association with that parental MHC background. As before, tolerance was assessed by testing the ability of LN cells from F1 donors pretreated with parental DNP-SC and then immunized to transfer contact sensitivity to naive parental strain recipients. Results are given in Table II. The top half shows results obtained from BALB/c parental recipients. When F1 donors are tolerized with BALB/c DNP-SC and then...
### Table II

**Selective Tolerance in F1 Mice of Lymphocyte Subsets Recognizing DNFB Associated with Parentally Derived MHC Antigens**

| Group | Pretreatment of F1 donor* | Recipient of Ft LN cells† | Ear swelling | Percentage of maximum response transferred |
|-------|--------------------------|---------------------------|--------------|-------------------------------------------|
| A     | Sham-BALB/c cells        | BALB/c                    | 30.4 ± 3.5   | 100                                       |
| B     | DNP-BALB/c cells         | BALB/c                    | 6.2 ± 0.7†   | 6†                                        |
| C     | Sham-CBA/J cells         | BALB/c                    | 26.5 ± 3.0   | 100                                       |
| D     | DNP-CBA/J cells          | BALB/c                    | 23.2 ± 1.4** | 94‡                                       |
| E     | —                        | BALB/c                    | 4.6 ± 1.2    | 0                                         |
| F     | Sham-CBA/J cells         | CBA/J                     | 41.8 ± 3.0   | 100†                                      |
| G     | DNP-CBA/J cells          | CBA/J                     | 20.2 ± 2.2†  | 35†                                       |
| H     | Sham-BALB/c cells        | CBA/J                     | 42.1 ± 1.4   | 100                                       |
| I     | DNP-BALB/c cells         | CBA/J                     | 34.1 ± 4.6** | 75‡                                       |
| J     | —                        | CBA/J                     | 8.6 ± 1.8    | 0                                         |

*Footnotes *, †, ‡, §, †, and ¶ are the same as in the legend in Table I.

**Not significantly different from sham-treated controls (P > 0.01).**

immunized, the immune LN cells transferred only 6% of the maximum response (compare groups B and A; P < 0.001). In contrast, immune LN cells from F1 donors that have been pretreated with CBA/J DNP-SC are not affected in their ability to transfer immunity to BALB/c recipients when compared with LN cells from sham-pretreated donors (compare groups D and C; P > 0.1). The reciprocal experiment, with CBA/J parental strain recipients, is shown in the bottom half of Table II. In this instance, tolerizing F1 donors with CBA/J DNP-SC, which did not affect the transfer of immunity to BALB/c recipients, significantly reduced the transfer of CBA/J recipients (65% inhibition) (compare groups G and F; P < 0.001). However, tolerizing F1 mice with BALB/c DNP-SC, which abrogated transfer to BALB/c recipients (94% inhibition; group D), did not significantly alter the ability of the LN cells to transfer contact sensitivity to CBA/J recipients (compare groups I and H; P > 0.1).

It is not clear why cells in the F1 donor recognizing antigen + H-2<sup>k</sup> are not totally inhibited by pretreatment with DNP-SC from CBA/J animals (group G). However, we have shown here that the transfer of immunity by DNFB-sensitized F1 donors is better into the CBA/J recipients than into the BALB/c parental strain. This difference in the transfer of immunity may be a result of a greater frequency of T cells in the F1 that recognize DNFB in association with the H-2<sup>k</sup> background than those recognizing the H-2<sup>d</sup> background. Thus, more DNP-SC from CBA/J animals may be required to tolerize all the reactive T cells. Nevertheless, our data show that pretreatment of the F1 donor with CBA/J DNP-SC significantly diminishes the ability of those LN cells to transfer contact sensitivity to CBA/J recipients.

Although the precise mechanism of tolerance induction in these animals is not known, we believe that it involves the interaction between the hapten-MHC antigen complex on the tolerogen and a T cell required for the expression of contact sensitivity. Furthermore, we have previously demonstrated that this regimen does not result in the generation of Ts. However, if Ts were induced by the intravenous injection of parental DNP-SC within the F1, they should suppress the transfer of sensitivity to both parental strains (9) and, thus, one would not find the selective T cell tolerance described here.
Summary

F1 animals were tolerized to 1-fluoro-2,4-dinitrobenzene (DNFB) contact sensitivity with parentally derived, in vitro hapten-modified spleen cells. This tolerant state was found, upon adoptive transfer to naive parental strain recipients, to affect only that T cell subpopulation that recognized the parental haplotype of the cell used as the tolerogen, and did not inhibit the ability of the remaining T cell subset to confer immunity. This demonstrates that this tolerant state involves the inactivation of a cell required for the expression of contact sensitivity by recognizing DNFB in association with self major histocompatibility complex gene products.

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