In recent years much interest has centered on the role of gene products of the major histocompatibility complex (MHC) in the immune response. The capacity to form antibodies to a variety of natural and synthetic antigens is under MHC-linked immune response (Ir) gene control in several mammalian species (1). Also a wide variety of effector and cooperative functions of thymus-derived lymphocytes (T cells) are associated with products coded by certain regions of the MHC. The I region is associated with the induction of helper T cells (2), the proliferation of T cells in response to allogeneic cells (3) and to antigen-coated macrophages (4), the cooperation of T helper cells from conventional mice with bone marrow-derived lymphocytes (B cells) (5), and the adoptive transfer of delayed-type hypersensitivity (DTH) to fowl gamma globulin (6). On the other hand, structures coded by the K or D regions of the MHC are associated with both specific T-cell-mediated cytolysis of lymphocytic choriomeningitis (LCM) virus-infected (7) or hapten-modified (8) target cell surfaces; and the transfer of DTH to murine LCM (9).

As yet there are no data indicating a similar MHC restriction on the generation of antigen-specific suppressor T cells (Ts). We have recently been studying the induction and mechanisms of tolerance to 1-fluoro-2,4-dinitrobenzene (DNFB) contact sensitivity using dinitrophenyl (DNP)-modified lymphoid cells (DNP-LC) as tolerogens (10). Mice injected with DNP-LC may appear tolerant (i.e., be "phenotypically tolerant") by virtue of one or the other (or both) of two mechanisms: (a) a rapidly induced, long-lasting, antigen-specific, cyclophosphamide (Cy)-insensitive period of inhibition of reactive T-cell clones (clone inhibition); and (b) a transient, antigen-specific, Cy-sensitive, infectious period of Ts activity (11). Thus, this system provided a unique opportunity to study possible genetic restrictions of Ts induction by testing the ability of a variety of DNP-modified syngeneic, semi-allogeneic, and allogeneic lymphoid cells to generate suppressor cells. We report here restriction of Ts induction to hapten-modified H-2 determinants.

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Materials and Methods

Mice. BALB/c mice were obtained from Simonsen Laboratories, Gilroy, Calif. CBA, C3H/HeJ, C3HBL/6, DBA/2, and A/J mice were obtained from The Jackson Laboratory, Bar Harbor, Maine.

Antigens and Tolerogens. DNFB was obtained from Sigma Chemical Co., St. Louis, Mo. Picryl chloride [1-chloro-2,4,6-trinitrobenzene (TNCB)] and picryl sulfonic acid were obtained from Matheson, Coleman & Bell, Cincinnati, Ohio.

Preparation of Hapten-Modified Lymphoid Cells. Erythrocyte-free spleen cell suspensions were prepared in Hanks' balanced salt solution (HBSS) as described previously (10). Spleen cells were dinitrophenylated exactly as previously described (10) and are termed DNP-LC. Spleen cells were trinitrophenylated by incubating equal volumes of spleen cells at 20 × 10^6/ml in HBSS and 10 mM picryl sulfonic acid (in HBSS) for 30 min at room temperature; these are termed TNP-LC. The haptenated cells were then pelleted, washed twice in HBSS, and adjusted to a concentration of 10^9/ml.

Induction of Tolerance. Mice were injected intravenously (i.v.) with 5 × 10^7 DNP-LC or TNP-LC in HBSS 7 days before contact sensitization or transfer of tolerance.

Sensitization and Elicitation of Contact Sensitivity. Mice were contact sensitized with DNFB or TNCB and the degree of sensitization determined by an ear thickness assay exactly as previously described (10). The increment in ear thickness is called "ear swelling" and is expressed in units of 10^-4 inches. Positive controls were sensitized and ear challenged; negative controls were ear challenged only. Percent tolerance was calculated according to the following formula:

\[
\% \text{ Tolerance} = \left[ \frac{\text{positive control} - \text{experimental}}{\text{positive control} - \text{negative control}} \right] \times 100.
\]

Transfer of Tolerance. Peripheral and mesenteric lymph nodes were collected 7 days after tolerization with 5 × 10^7 DNP or TNP-modified lymphoid cells. Single cell suspensions were prepared in HBSS and 100 × 10^6 donor lymphocytes were injected i.v. into either normal or 250 R irradiated syngeneic recipients (12). Control mice received either no cells or cells from normal donors. The recipient and control mice were contact sensitized 1–2 h after transfer. The degree of tolerance in recipient mice was expressed as percent tolerance transferred as described above.

Results and Discussion

We approached the problem by examining the ability of a wide variety of DNP-LC to (a) induce tolerance in donor animals (phenotypic tolerance) and to (b) generate transferable Ts. Table I shows that DNP on syngeneic, semiallogeneic, or allogeneic mouse lymphoid cells can induce nearly complete tolerance in BALB/c mice. These data contrast with results from the same mice as to the ability of the hapten-modified cells to generate suppressor T cells. It is clear that 100 × 10^6 lymph node cells from BALB/c (H-2^d) mice tolerized with syngeneic DNP-LC (group A) transferred a significant level of tolerance to syngeneic recipients. However, hapten-modified allogeneic mouse cells uniformly failed to generate suppressor cells effective in syngeneic recipients (groups B–D). Group E shows that DBA/2 DNP-LC, compatible at the MHC locus with BALB/c mice but not compatible outside it, can also generate suppressor cells. Group F shows that recombinant A/J (kkkddd) DNP-LC were able to generate suppressor cells at a level not significantly different from the syngeneic system and indicates that identity between the DNP/LC tolerogens and the donor mice at the Ic, Ss, and D loci is sufficient for Ts induction.

It was essential to establish that mice possessing other than H-2^d cell surface antigenic specificities were capable of generating suppressor cells to hapten-modified lymphoid cells. This was accomplished by comparing the ability of both BALB/c (H-2^d) and CBA (H-2^k) DNP-LC to induce Ts in the reciprocal strains.
Table I

**H-2 Restriction of Suppressor T-Cell Induction to DNP-Modified Lymphoid Cells in BALB/c Mice**

| Group | DNP-LC tolerogen* | Group | DNP-LC tolerogen* |
|-------|-------------------|-------|-------------------|
|       | H-2 haplotype     |       | H-2 haplotype     |
|       | Strain | K | la | lb | ic | Ss | D |       | Strain | K | la | lb | ic | Ss | D |
| A     | BALB/c | d | d | d | d | d | d | 96.1 ± 3.1 | 59.1 ± 6.3 |
| B     | CBA | k | k | k | k | k | k | 90.6 ± 2.8 | -4.2 ± 6.5§ |
| C     | C3H/HeJ | k | k | k | k | k | k | 98.4 ± 2.8 | 6.0 ± 2.11 |
| D     | C57BL/6 | b | b | b | b | b | b | 82.4 ± 2.8 | 0.3 ± 1.21 |
| E     | DBA/2 | d | d | d | d | d | d | 96.1 ± 5.3 | 59.1 ± 3.5 |
| F     | A/J | k | k | k | d | d | d | 93.2 ± 6.3 | 62.5 ± 3.9 |

* BALB/c mice were tolerized with 5 × 10⁷ DNP-LC suppressor T-cell induction.

§ Not significant, P > 0.10.

Table II

**Demonstration of Reciprocal H-2 Restriction of Suppressor T-Cell Induction to DNP-Modified Lymphoid Cells in BALB/c and CBA Mice**

| DNP-LC tolerogen* (H-2 haplotype) | Tolerized strain (H-2 haplotype) | % Phenotypic tolerance in tolerized strain | % Tolerance transferred from tolerized strain to syngeneic recipient* |
|----------------------------------|----------------------------------|------------------------------------------|---------------------------------------------------------------|
| BALB/c DNP-LC (ddddddd)          | BALB/c (ddddddd)                 | 87.5 ± 4.74                              | 68.1 ± 7.15                                                   |
| CBA DNP-LC (kkkkkk)             | CBA (kkkkkk)                     | 84.1 ± 7.51                              | -2.1 ± 7.7                                                    |
| BALB/c DNP-LC (ddddddd)          | CBA (kkkkkk)                     | 81.9 ± 5.11                              | -0.4 ± 5.1                                                    |
| CBA DNP-LC (kkkkkk)             | CBA (kkkkkk)                     | 86.1 ± 3.31                              | 58.7 ± 1.75                                                   |

* Mice were tolerized with 5 × 10⁷ DNP-BALB/c or DNP-CBA lymphoid cells 7 days before assay of phenotypic tolerance and suppressor T-cell induction.

§ Mice received 100 × 10⁶ lymph node cells from tolerant BALB/c donors and CBA mice the same number of cells from CBA donors.

§ Significant, P < 0.001.

Table II shows that Ts induction was restricted to the syngeneic DNP-modified lymphoid cells in both strains. BALB/c mice generated Ts only to BALB/c DNP-LC and CBA mice only to CBA DNP-LC, but each strain became phenotypically unresponsive after tolerization with either syngeneic or allogeneic DNP-LC.

To determine if H-2 restriction of suppressor cell induction could be demonstrated with another contactant; i.e., TNCB (picryl chloride), BALB/c mice were doubly tolerized with 5 × 10⁷ DNP- and 5 × 10⁷ TNP-modified lymphoid cells on either syngeneic (BALB/c) or allogeneic (CBA) backgrounds. The results are shown in Table III and illustrate that mice tolerized with BALB/c DNP-LC plus CBA TNP-LC were phenotypically tolerant to both contactants but transferred tolerance only to DNFB contact sensitization (exp. 1). The reciprocal experiment (Table III, exp. 2) shows that BALB/c mice tolerized with BALB/c TNP-LC plus CBA DNP-LC were phenotypically tolerant to both contactants but generated Ts only to the hapten presented on the H-2 compatible background, in this case TNP.

The studies described above illustrate two major points. First, mice can be made phenotypically tolerant to either DNFB or TNCB contact sensitization by
using the appropriate hapten-modified lymphoid cells from syngeneic or allogeneic mouse strains. Second, and most important, Ts in this system are generated by recognition of hapten-modified H-2-specified cell surface determinants. We have also shown that identity at the right end of the MHC is sufficient for suppressor cell induction in the DNFB system (Table I).

It has been amply illustrated in other systems that T cells mediating both cytotoxic and suppressive functions bear the Ly 2,3 phenotype (13, 14). It has also been shown that killer T-cell-mediated cytolysis of hapten-modified (8) and virus-infected (7) target cells requires recognition of modified K or D cell surface determinants. Thus, one could speculate that cells bearing the Ly 2,3 phenotype respond to hapten or antigen-modified K or D determinants whether they are mediating killer or suppressor functions. Proof of this awaits determination of the Ly phenotype of the Ts in our system and the fine mapping of the hapten-modified MHC region(s) responsible for Ts induction.

The results suggest that phenotypic tolerance to DNFB contact sensitization can be achieved by DNP-modified allogeneic or syngeneic cell membranes via inhibition of reactive T-cell clones. This probably occurs via direct receptor blockade with the DNP-membrane grid, but not with DNP-soluble self carriers (10) which are potent B-cell tolerogens (15). In contrast to clone inhibition, Ts precursors are activated by recognition of DNP on an H-2 compatible background. It is also possible that DNP-modified allogeneic lymphoid cells might induce Ts directed against the DNP-allogeneic H-2 background. If this were correct, the suppressor cells would have to be assayed in the allogeneic strain. In the present study, suppressor cells were assayed only in the strain used for their induction. This is under investigation.

**Summary**

Studies using hapten-modified lymphoid cells as tolerogens for 1-fluoro-2,4-dinitrobenzene contact sensitization have shown that BALB/c (H-2^b^) mice can be made phenotypically tolerant by dinitrophenyl (DNP) on either syngeneic or allogeneic mouse lymphoid cells (DNP-LC). However, suppressor T-cell induc-
tion (Ts) in these mice (as demonstrated by adoptive transfer to syngeneic recipients) was restricted to \(H-2\) identity between the DNP-LC and the donor mouse. It was also shown that identity at the right end of the \(H-2\) complex was sufficient for Ts induction. In addition, this restriction was also demonstrated in CBA (\(H-2^b\)) mice and for tolerance in the 1-chloro-2,4,6-trinitrobenzene contact sensitivity system using trinitrophenyl-modified lymphoid cells.

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