GENETIC RESTRICTIONS FOR THE INDUCTION OF SUPPRESSOR T CELLS BY HAPTN-MODIFIED LYMPHOID CELLS IN TOLERANCE TO 1-FLUORO-2,4-DINITROBENZENE CONTACT SENSITIVITY.

Role of the H-2D Region of the Major Histocompatibility Complex*

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Gene products of the major histocompatibility complex (MHC)† are associated with various effector and cooperative functions of immunocompetent lymphoid cells (1). The I region of the MHC is associated with the induction of helper T cells (2), the proliferative response of T cells to allogeneic cells (3) and to antigen-pulsed macrophages (4), cooperation of T helper cells with B cells (5), and the adoptive transfer of delayed-type hypersensitivity (DTH) to fowl gamma globulin (6). Gene products of the H-2K and/or H-2D regions of the MHC are associated with T-cell-mediated cytolysis of virus-infected (7) or hapten-modified (8) target cells, and the transfer of DTH to murine lymphocytic choriomeningitis virus (9).

We have recently been studying the induction and mechanisms of hapten-specific T-cell tolerance to 1-fluoro-2,4-dinitrobenzene (DNFB) contact sensitivity by using dinitrophenyl (DNP)-modified lymphoid cells (DNP-LC) as tolerogens (10). Tolerance induced by DNP-LC has been functionally separated into two mechanisms—clone inhibition and the participation of suppressor T cells (Ts) (11). We have also reported that the induction of Ts by DNP-syngeneic LC (syninduced Ts) (12) as well as the expression of Ts induced by DNP-allogeneic LC (alloinduced Ts) (13) is genetically restricted by MHC gene products. In the present report, we have investigated these genetic restrictions by using congenic resistant mouse strains. The results indicate that both syninduced and alloinduced Ts are activated by hapten-modified determinants coded for by the H-2D region of the MHC. Furthermore, the results show that alloinduced Ts are

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‡ Abbreviations used in this paper: Cy, cyclophosphamide; DNBSO₃, 2,4-dinitrobenzene sulfonate; DNFB, 1-fluoro-2,4-dinitrobenzene; DNP, 2,4-dinitrophenyl; DNP-LC, DNP-lymphoid cells; DTH, delayed type hypersensitivity; HBSS, Hanks' balanced salt solution; LC, lymphoid cells; LN, lymph node; LNC, lymph node cells; MHC, major histocompatibility complex; Ts, suppressor T cells; Tc, cytotoxic T cells; TNCB, 2,4,6-trinitrobenzene.
suppressive only when they are transferred to recipient mice which share H-2D region determinants with the DNP-LC donor.

Materials and Methods

Mice. BALB/c mice were obtained from Simonsen Laboratories, Gilroy, Calif. A/J, B10.BR, B10.D2, C3H/HeJ, C57Bl/6, CBA, DBA/2, and SJL mice were obtained from The Jackson Laboratory, Bar Harbor, Maine. A.TH, A.TL, and B10.A(4R) mice were kindly provided by Dr. J. W. Moorhead, University of Colorado Medical Center.

Antigens. DNP was obtained from Sigma Chemical Co., St. Louis, Mo.

Preparation of Hapten-Modified Lymphoid Cells. Erythrocyte-free spleen and lymph node cell suspensions were prepared in Hanks' balanced salt solution (HBSS) and were dinitrophenylated as described previously (10). These preparations are termed DNP-LC. After hapten-modification, the DNP-LC were washed twice in HBSS, and adjusted to a concentration of 10⁸/ml.

Induction and Transfer of Tolerance. Normal mice were injected intravenously (i.v.) with 5 x 10⁷ DNP-LC in HBSS 7 days before transfer of tolerance. On day 0, peripheral and mesenteric lymph nodes (LN) were collected from the tolerant mice (Ts donors) and single cell suspensions were prepared in Mishell-Dutton balanced salt solution (BSS). 100 x 10⁷ LN cells were injected i.v. into normal or 250 rads irradiated (³²Co) recipients (14). Control mice received either no cells or 100 x 10⁷ LN cells from donors injected with sham-labeled LC. This experimental protocol is illustrated in Fig. 1.

Assay of Suppressor T-Cell Induction. Within 1 h after cell transfer, recipient mice were contact sensitized with 0.5% DNFB in 4:1 acetone:olive oil on 2 successive days. 4 days after the last paint, the mice were challenged on the ears with 0.2% DNFB and the degree of sensitization determined 24 h later by measuring increased ear swelling with an engineer's micrometer (11). The increment in ear thickness is expressed in units of 10⁻⁴ inches. The percentage of tolerance transferred was calculated by comparing the response of Ts recipient mice to that of positive (sensitized and ear challenged) and negative (ear challenged only) control mice:

\[
\% \text{ tolerance transferred} = \left(\frac{\text{positive control} - \text{experimental}}{\text{positive control} - \text{negative control}}\right) \times 100.
\]

Results

Genetic Restrictions for the Induction of Suppressor T Cells Which Are Nongenetically Restricted for Suppression (Syninduced Ts). We have previously shown that Ts active in suppressing DNFB contact sensitivity can be induced by the injection of DNP-modified syngeneic lymphocytes. These syninduced Ts are active in mediating hapten-specific suppression in recipient mice regardless of the recipients genetic background. That is, DNP-BALB/c lymphoid cells can induce Ts in BALB/c donor mice which are able to transfer suppression to BALB/c and to CBA recipients (13). To investigate the genetic restrictions for induction of syninduced Ts, various mouse strains were tolerized with DNP-LC which shared with the Ts donors either H-2 or non-H-2 genetic material. Suppressor cell induction was assayed by determining the ability of 10⁸ lymph node cells (LNC) from the Ts donor strains to transfer hapten-specific suppression to MHC-compatible BALB/c recipient mice.

The results of these experiments are shown in Table I. Tolerization of BALB/c mice with syngeneic DNP-BALB/c (group A) or MHC compatible DNP-B10.D2 (group B) LC led to the induction of Ts which, when transferred to BALB/c recipients, caused significant suppression of the DNFB contact sensitivity response. On the other hand, tolerization of B10.D2 mice with DNP-B10.BR LC (group C), which share non-H-2 genetic material with the B10.D2 donors, fails
to induce Ts capable of suppressing BALB/c recipients. Group D reconfirms the observation that we have previously reported (12) that tolerization of BALB/c mice with allogeneic DNP-CBA LC does not cause the induction of Ts active in BALB/c recipients. Thus, identity at the MHC between the DNP-LC tolerogen and the Ts donor strain is required for the induction of syninduced Ts.

Having demonstrated an MHC compatibility requirement for the induction of syninduced Ts, it was of interest to determine which region(s) of the MHC on the hapten-modified lymphoid cells were involved in Ts induction. To examine
TABLE II

Genetic Restrictions Governing the Induction of Syninduced Suppressor T Cells Map to the H-2D Region of the MHC

| Experimental group | DNP-LC Tolerogen (K I D)* | Ts Donor strain (K I D) | MHC Identity between DNP-LC tolerogen and Ts donor strain | Recipient strain (K I D) | % Tolerance transferred ||
|-------------------|--------------------------|------------------------|------------------------------------------------------|------------------------|-----------------||
| A-1               | DNP-A/J (k k/d d)        | BALB/c (d d d)         | IC → D                                               | BALB/c (d d d)         | 62.6 ± 3.9%     ||
| 2                 | DNP-A/J (k k/d d)        | CBA (k k k)            | K → IB                                              | CBA (k k k)            | 4.7 ± 1.6       ||
| B-1               | DNP-A.TL (s k d)         | CBA (k k k)            | IA → G                                              | CBA (k k k)            | 2.7 ± 4.6       ||
| 2                 | DNP-A.TL (s k d)         | BALB/c (d d d)         | D                                                   | BALB/c (d d d)         | 58.3 ± 4.8%     ||
| 3                 | DNP-A.TH (s s d)         | BALB/c (d d d)         | D                                                   | BALB/c (d d d)         | 57.8 ± 4.5%     ||

* Haplotype of the K, I, and D regions of the H-2 complex.
† Ts Donor mice were tolerized with 5 × 10⁶ DNP-LC of the indicated backgrounds on day -7.
‡ Recipients were normal mice who received 10⁶ LNC from the tolerant donors on day 0.
§ Mean values for two to four experiments ± SEM.
¶ Values significant P < 0.01.

This question, various mouse strains were tolerized with DNP-LC whose haplotypes shared all or portions of the MHC with the tolerized mice (Ts donor strains). Suppressor cells were measured by determining the ability of the Ts donor strains to transfer hapten-specific suppression to syngeneic recipient mice. The results are shown in Table II.

We first determined the ability of DNP-A/J (H-2a) LC to induce Ts in BALB/c and CBA donor mice. Group A-1 shows that BALB/c mice tolerized with DNP-A/J LC (IC → D region compatible) were able to transfer significant suppression to BALB/c recipients; however, CBA mice tolerized with DNP-A/J LC (K → IB region compatible) were incapable of transferring suppression to syngeneic CBA recipients (group A-2). Thus, compatibility in the right half of the MHC (IC → D regions) between the DNP-LC tolerogen and the Ts donor was sufficient and required for Ts induction, whereas compatibility in the left half of the MHC apparently played no role in this process.

To further define the hapten-modified MHC region involved in Ts induction, lymphoid cells from various congenic recombinant mice were dinitrophenylated in vitro and used to tolerize donor mice of varying H-2 haplotypes. LN cells from these tolerant donors were transferred to syngeneic recipients to assay for Ts induction. CBA mice injected with DNP-A.TL LC (IA → G region compatible) did not contain Ts active in CBA recipients (group B-1). Thus, I region compatibility alone was not sufficient for the induction of syninduced Ts. However, when BALB/c mice were tolerized with DNP-A.TL (group B-2) or DNP-A.TH (group B-3) LC, H-2D region identical only, they were found to be as efficient in transferring suppression to BALB/c recipients as were donors...
H-2D REGION INVOLVEMENT IN Ts INDUCTION

Table III

Demonstration of MHC Restriction for the Induction and Expression of Alloinduced Suppressor T Cells

<table>
<thead>
<tr>
<th>Group</th>
<th>DNP-LC Tolerogen</th>
<th>Ts Donor* strain</th>
<th>Recipient† strain</th>
<th>Compatibility between DNP-LC tolerogen and recipient strain</th>
<th>% Tolerance transferred‡</th>
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</thead>
<tbody>
<tr>
<td>A</td>
<td>DNP-B10. BR (H-2*)</td>
<td>B10. D2 (H-2*)</td>
<td>B10. BR (H-2*)</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>B</td>
<td>DNP-B10. BR (H-2*)</td>
<td>B10. D2 (H-2*)</td>
<td>CBA (H-2*)</td>
<td>+</td>
<td>-</td>
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<tr>
<td>C</td>
<td>DNP-B10. BR (H-2*)</td>
<td>B10. D2 (H-2*)</td>
<td>B10. D2 (H-2*)</td>
<td>-</td>
<td>+</td>
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<tr>
<td>D</td>
<td>DNP-B10. BR (H-2*)</td>
<td>B10. D2 (H-2*)</td>
<td>BALB/c (H-2*)</td>
<td>-</td>
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</table>

* B10. D2 (H-2*) Ts Donor mice were tolerized with 5 × 10⁷ DNP-B10. BR (H-2*) LC on day -7.
† Recipients were normal mice who received 10⁸ LNC from the tolerant donors on day 0.
‡ Mean values for four to five mice per experimental group ± SEM.
§ Values significant P < 0.01.

Genetic Restrictions for the Induction and Expression of H-2-Restricted Suppressor T Cells (Alloinduced Ts). Mice can be made tolerant to DNFB contact sensitivity by injection of either DNP-syngeneic or DNP-allogeneic lymphoid cells (12). Suppressor cells induced by DNP-allogeneic lymphoid cells (alloinduced Ts) are active only when transferred to the specific allogeneic strain (i.e., Ts induced by injecting DNP-CBA LC into BALB/c mice can only suppress DNFB contact sensitivity in CBA recipients, not in BALB/c or third party recipients) (13). Therefore genetic requirements for both the induction and expression of alloinduced Ts were investigated.

We first determined whether alloinduced Ts could be raised in B10. D2 (H-2*) Ts donor mice tolerized with DNP-B10. BR (H-2*) LC. These strains are H-2 incompatible but share non-MHC genetic material. Ts induced in this manner were then assayed in various recipient strains which shared H-2 and/or non-H-2 genetic material with the DNP-B10. BR tolerogen. The results are shown in Table III. Suppression was only observable in recipient mice which were syngeneic (B10. BR−group A) or MHC compatible (CBA−group B) with the tolerogen. Recipients which were allogeneic at the H-2 complex, but shared non-MHC regions (B10. D2−group C) or which were completely allogeneic (BALB/c−group D) to the tolerogen were not suppressed. Thus, alloinduced Ts can be induced in DNP-LC tolerogen-Ts donor strain combinations which differ only within the H-2 complex. Also, expression of these alloinduced Ts is restricted to recipient mice which share the MHC region with the DNP-LC tolerogen used for their induction. Compatibility in non-MHC regions appears
We next examined which MHC region determinant(s) were involved in both the induction and expression of alloinduced Ts. Various mouse strains were tolerized on day -7 with 5 x 10^7 DNP-LC. The haplotypes of the DNP-LC differed at all or various portions of the MHC from the tolerized mice (Ts donor strains). This constituted the Ts induction phase of the experiments. Ts expression was assayed by examining the ability of the Ts donor strains to transfer hapten-specific suppression to recipient mice of varying H-2 haplotypes. The results of these experiments are shown in Table IV.

CBA mice tolerized with DNP-A/J LC (incompatible at the \( IC \rightarrow D \) regions) were able to transfer suppression to BALB/c (group A-2) recipients (compatible with the DNP-A/J LC tolerogen at the \( IC \rightarrow D \) regions), but not to CBA recipients (group A-1), which are compatible at the \( K \rightarrow IE \) regions with the tolerogen. The converse experiment is shown in group B and yielded comparable results. A/J mice were tolerized with DNP-CBA LC (incompatible at the \( IC \rightarrow D \) regions) and were found to transfer suppression to CBA recipients (group B-1) which are syngeneic to the DNP-CBA tolerogen. These donors were unable to transfer suppression to BALB/c (group B-2) or A/J (group B-3) recipients who share none or the \( K \rightarrow IE \) regions, respectively, with the tolerogen. The above

### Table IV  
Genetic Restrictions Governing the Induction and Expression of Alloinduced Suppressor T Cells Map to the D Region of the MHC

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>DNP-LC Tolerogen (K I D)*</th>
<th>Ts Donor strain (K I D)</th>
<th>MHC Identity between DNP-LC tolerogen and Ts donor strain</th>
<th>Recipient strain (K I D)</th>
<th>MHC Identity between DNP-LC tolerogen and recipient strain</th>
<th>% Tolerance transferred</th>
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<tbody>
<tr>
<td>A-1</td>
<td>DNP-A/J (k k/d d)</td>
<td>CBA (k k k)</td>
<td>IE (\rightarrow) D</td>
<td>CBA (k k k)</td>
<td>K (\rightarrow) IE</td>
<td>71.1 \pm 6.1**</td>
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<td></td>
<td>BALB/c (d d d)</td>
<td>K (\rightarrow) D</td>
<td>71.1 \pm 6.1**</td>
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<td>B-1</td>
<td>DNP-CBA (k k k)</td>
<td>A/J (k k/d d)</td>
<td>IC (\rightarrow) D</td>
<td>CBA (k k k)</td>
<td>All</td>
<td>51.0 \pm 2.9**</td>
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<td>BALB/c (d d d)</td>
<td>None</td>
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<td></td>
<td>A/J (k k/d d)</td>
<td>K (\rightarrow) IE</td>
<td>4.5 \pm 3.3</td>
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<tr>
<td>C-1</td>
<td>DNP-B10.A(4R) (k k/b b)</td>
<td>BALB/c (d d d)</td>
<td>All</td>
<td>CBA (k k k)</td>
<td>K (\rightarrow) IA</td>
<td>-3.0 \pm 3.7</td>
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<td></td>
<td>CSTBL/6 (b b b)</td>
<td>IB (\rightarrow) D</td>
<td>66.7 \pm 8.3**</td>
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<td>D-1</td>
<td>DNP-A.TH (s a d)</td>
<td>SJL (s a s)</td>
<td>D</td>
<td>BALB/c (d d d)</td>
<td>D</td>
<td>78.7 \pm 4.7**</td>
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<td></td>
<td>SJL (s a s)</td>
<td>K (\rightarrow) G</td>
<td>-4.2 \pm 4.7</td>
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</table>

* Haplotype of the K, I, and D regions of the H-2 complex.  
† Ts Donor mice were tolerized with 5 x 10^7 DNP-LC of the indicated backgrounds on day -7. 
‡ Recipients were normal mice who received 10^6 LNC from the tolerant donors on day 0. 
§ Mean values for two to four experiments \(\pm\) SEM. 
¶ Values significant \(P < 0.01\).
H-2D REGION INVOLVEMENT IN Ts INDUCTION

Two experiments bring out two points—first, incompatibility within the right half of the MHC is sufficient for induction of alloinduced Ts; and, alloinduced Ts can suppress recipient mice only if they share at least right half (IC $\rightarrow D$ regions) $H-2$ compatibility with the DNP-LC used for their induction. The latter point is again illustrated in group C. BALB/c mice tolerized with DNP-B10.A(4R) LC were found to transfer suppression only to C57BL/6 recipients (group C-2) which share IC $\rightarrow D$ regions with the tolerogen. These Ts were not capable of suppressing CBA recipients (group C-1) which share $K + IA$ regions with the DNP-B10.A(4R) tolerogen. Group C-1 also shows that incompatibility at the $K + IA$ regions between the DNP-LC tolerogen and the Ts donor strain does not induce Ts active in recipients displaying compatible $K + IA$ regions with the tolerogen.

To further define the region(s) of the MHC which is involved in both the induction and expression of alloinduced Ts, SJL ($H-2^s$) mice were tolerized with DNP-A.TH LC. These strains differ only at the $H-2D$ region of the MHC. Tolerant cells were then transferred to BALB/c and SJL recipient mice. Suppression was observed in BALB/c (group D-1), but not in SJL (group D-2) recipients. Thus, incompatibility at only the $H-2D$ region is both sufficient and required for the induction of alloinduced Ts. These Ts are capable of suppressing BALB/c recipients which share only the $H-2D$ region with the DNP-LC tolerogen used for their induction, but can not suppress SJL recipient mice which share all but the $H-2D$ region of the MHC with the tolerogen.

Discussion

The understanding of immunoregulation requires knowledge of the genetic restrictions of such regulation. The DNFB contact sensitivity model is highly appropriate as it is simple to induce a hapten-specific, T-cell-mediated delayed hypersensitivity reaction merely by a topical application of DNFB on the skin. Hapten-specific, T-cell-mediated tolerance in this model is induced by parenteral injection of either DNFB, 2,4-dinitrobenzene sulfonate (DNBSO$_3$) or in vitro DNP-modified lymphoid cells (10, 15). Tolerance induced by DNP-LC has been shown to be mediated by dual mechanisms (11): a rapidly induced, long lasting, antigen-specific, cyclophosphamide (Cy)-insensitive period of inhibition of reactive T-cell clones (clone inhibition); and, a transient, antigen-specific, Cy-sensitive, infectious period of Ts activity. The ability to generate suppressor T cells by injecting hapten-modified lymphoid cells, as opposed to free hapten, has allowed us to study the genetic requirements for the induction and expression of Ts (12, 13).

In a previous report (12), it was shown that injection of DNP-syngeneic LC into BALB/c mice made those mice tolerant to DNFB contact sensitization and generated Ts able to transfer tolerance into normal BALB/c recipients. In the present report, the use of congenic mouse strains has shown that MHC compatibility alone is both sufficient and required for the induction of syninduced Ts (Table I). Interestingly, Ts induced in this manner (syninduced Ts) are not genetically restricted in their expression, and will suppress allogeneic recipients (13). In contrast, injection of DNP-allogeneic LC into BALB/c mice, although rendering those mice tolerant to DNFB, did not induce Ts which were
able to suppress BALB/c recipients (12). Rather, tolerization with DNP-allogeneic LC induced Ts which were active only in the strain which provided the DNP-LC tolerogen (13). For example, BALB/c mice tolerized with DNP-CBA LC transferred suppression only to CBA recipients. We have termed these latter cells alloinduced Ts. In the present report, we have also shown that alloinduced Ts can be generated in DNP-LC tolerogen-Ts donor combinations which differ only within the H-2 complex. Further, the expression of these Ts has been shown to be restricted to recipient mice which share MHC region genes with the DNP-LC tolerogen used for Ts induction (Table III).

Having demonstrated the above mentioned H-2 restrictions for the induction of syninduced Ts and the induction and expression of alloinduced Ts, we investigated the hapten-modified MHC regions involved in these restrictions. The results indicate that the induction of both syninduced and alloinduced Ts and the expression of alloinduced Ts is associated with determinants coded for by the H-2D region of the MHC. When the DNP-LC tolerogen and the Ts donor mouse are H-2 compatible only within the H-2D region, syninduced Ts are induced which can transfer suppression to recipient mice syngeneic with the Ts donor strain (Table II). This was most clearly illustrated by experiments showing that DNP-A.TL or DNP-A.TH LC caused the induction of Ts in BALB/c mice (H-2D region compatibility) able to suppress BALB/c recipients. As previously reported, these syninduced Ts are hapten-specific, but non-H-2 restricted in their expression. In contrast, when the DNP-LC tolerogen and the Ts donor strain are incompatible at the H-2D region of the MHC, alloinduced Ts are activated which can transfer suppression to recipient mice which share only H-2D region compatibility with the tolerogen, but not to recipients sharing other MHC regions with the tolerogen. Thus, hapten modification of determinants coded for by the H-2D region is sufficient for the induction of alloinduced Ts, and identity at this same determinant is required for their expression in recipient mice. This is illustrated by the fact that DNP-A.TH LC caused the induction of H-2-restricted Ts in SJL mice capable of transferring suppression to BALB/c, but not to SJL recipients (Table IV—group D). By H-2D region determinants, we mean determinants coded for by that part of the MHC which maps to the right of the H-2G locus. It is possible that the region in question lies between the H-2G and the H-2D loci.

The association of Ts functions with regions in the right half of the MHC has been reported in other experimental systems. Rich and Rich have described a system wherein a suppressor factor liberated by alloantigen-activated splenic T cells can inhibit mixed lymphocyte responses only of responder cells of those strains that share IC and S subregions of the H-2 complex with the cells producing the factor (16, 17). Pang and Blanden (18) have also shown that splenic suppressor T cells from ectromelia virus infected mice could suppress the response of recipient mice to the virus. H-2D region compatibility between donor and recipient mice was sufficient for suppression to be expressed, but I region compatibility was neither sufficient or necessary. More recently, Kumar and Bennett (19) have shown that the interaction between suppressor T cells and mitogen-responsive cells in cultures infected with Friend leukemia virus required identity at the H-2D subregion.
Interestingly, the association of both \textit{H-2K} and \textit{H-2D} regions of the MHC have been described for both positive and negative aspects of DNFB contact sensitivity. Vadas et al. (20) have recently shown that contact sensitivity to DNFB can be successfully transferred between donor and recipient mouse strains which share only the \textit{H-2K} and/or \textit{H-2D} regions of the MHC. It should be pointed out that this association does not hold in all DTH systems, since \textit{I} region compatibility is required for transfer of DTH to fowl gamma globulin (6, 20). In terms of tolerance to DNFB contact sensitivity, Phanuphak et al. have shown that tolerization with DNBSO\textsubscript{3} leads to the induction of hapten-specific Ts (21) which block the afferent limb of sensitization by inhibiting antigen-induced DNA synthesis (14). These Ts carry surface-associated tolerogen (22) and are non-H-2 restricted in suppression. However, a soluble suppressor factor liberated in vitro by these cells inhibits the effertent limb of the response by blocking the passive transfer of DNFB contact sensitivity by immune LNC (23). This factor has been shown to be an \textit{I} region product, and will only block immune LNC which share \textit{H-2K} and/or \textit{H-2D} region homology with the factor producing strain (24).

With regard to alloinduced Ts, they seem to be induced by recognizing DNP-modified-\textit{H-2D} region determinants on the allogeneic DNP-LC tolerogen. Their genetic restriction is understandable in that they only suppress recipient mice which share the \textit{H-2D} region with the DNP-LC tolerogen and in whom allogeneic DNP-\textit{H-2D} region determinants are generated by subsequent contact sensitization with DNFB. We have recently determined that similar to the DNBSO\textsubscript{3} induced Ts, alloinduced Ts inhibit afferent sensitization and carry surface-associated DNP.\textsuperscript{2} The situation with syninduced Ts is vastly different. They seem to be activated by recognition of DNP-modified-self-\textit{H-2D} region determinants. Unlike alloinduced Ts, syninduced Ts are non-MHC restricted, carry no detectable surface-associated DNP, and do not inhibit afferent sensitization, but rather block the passive transfer of contact sensitivity by DNFB immune LNC.\textsuperscript{4} In this regard, they are similar to the Ts described by Asherson et al. (25) induced by the injection of TNBSO\textsubscript{3} which block the passive transfer of sensitivity by trinitrochlorobenzene-immune LNC. Whether syninduced Ts are suppressive by virtue of competing with immune LNC for the immunogenic form of DNP on the surface of stimulator macrophages or by direct effects on immune LNC mediated by direct contact or through soluble suppressor factors is not yet known and is currently under active investigation. Also, whether syninduced Ts are non-MHC restricted by virtue of cross-reactive T-cell receptors which can recognize hapten-modified allogeneic determinants is at this time not clear.

It has been amply illustrated in other systems that T cells mediating both cytotoxic and suppressive functions bear the Ly 2,3 phenotype (26, 27). It has also been shown that T-cell-mediated cytolysis of hapten-modified (8) and virus-infected (7) target cells requires recognition of modified \textit{H-2K} or \textit{H-2D} cell surface determinants. In this regard, syninduced suppressor T cells in our system which are activated by hapten-modified self lymphoid cells, differ from cytotoxic T cells (Tc) directed against hapten-modified self targets, in that Ts

\textsuperscript{1} S. D. Miller, M-S. Sy, and H. N. Claman. 1978. Manuscript in preparation.
induction requires \( H-2D \) region compatibility, but Tc are capable of recognizing modified \( H-2K \) or \( H-2D \) determinants. This dichotomy may reflect differences in the sensitivity of the two assay systems. More importantly, it may reflect fundamental differences in the hapten-modified \( H-2 \) region determinant(s) which cause activation of the Ts or Tc subpopulations. The Ly phenotypes of syninduced and alloinduced Ts in this system are not known. Even if they both carry Ly 2,3 antigens, the complexity of the system is illustrated by the fact that both cells appear to recognize DNP-modified \( H-2D \) products yet one works on the afferent limb and the other on the efferent limb.

Summary

Genetic restrictions governing the induction and expression of suppressor T cells (Ts) in tolerance to 1-fluoro-2,4-dinitrobenzene (DNFB) contact sensitivity were studied. Tolerance was induced by using 2,4-dinitrophenyl (DNP)-modified lymphoid cells (DNP-LC) as tolerogen. Two kinds of Ts were found—those produced by DNP-LC syngeneic to the donor of the Ts (syninduced Ts), and those produced by DNP-LC allogeneic to the donor of Ts (alloinduced Ts). Studies employing congenic resistant mouse strains indicated that recognition of DNP-modified-major histocompatibility region determinants on the tolerogenic DNP-LC was essential for the induction of both types of Ts. Non-H-2 genetic background was irrelevant to Ts induction. Mapping studies indicated that induction of both syninduced and alloinduced Ts was associated with recognition of DNP-modified-MHC region determinants which map to the right of the \( H-2G \) region (i.e., \( H-2D \) gene products). Tolerization of donor mice with DNP-LC which were \( H-2D \) region compatible, but not with \( H-2K \) or I region compatible DNP-LC, was both sufficient and required for the induction of hapten-specific syninduced Ts. Tolerization of donor mice with DNP-LC which were incompatible only at the \( H-2D \) region was sufficient for the induction of alloinduced Ts. These Ts were capable of suppressing recipient mice only if the recipients shared the \( H-2D \) region with the strain providing the DNP-LC tolerogen, and were not capable of suppressing recipients sharing all but the \( H-2D \) region with the tolerogen.

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References

H-2D REREGION INVOLVEMENT IN Ts INDUCTION


