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Impaired Cutaneous Immune Responses in Thy-1-Deficient Mice

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Thy-1 is a cell surface glycoprotein expressed mainly on brain and lymphoid tissue. Although the functions of Thy-1 are incompletely understood, evidence exists that Thy-1 participates in T cell activation. To examine the functional role of Thy-1 in cutaneous immune responses in vivo, Thy-1 gene-targeted mice (Thy-1<sup>−/−</sup>) and wild-type mice (Thy-1<sup>+/+</sup>) were immunized with the hapten oxazolone. After challenge with oxazolone, contact hypersensitivity responses in Thy-1<sup>−/−</sup> mice were reduced by 25% compared with Thy-1<sup>+/+</sup> mice. Likewise, irritant dermatitis induced by croton oil was also decreased. In addition, Thy-1<sup>−/−</sup> mice showed a significantly reduced delayed-type hypersensitivity response after injection of allogeneic spleen cells into the hind footpads of allosensitized animals when compared with Thy-1<sup>+/+</sup> mice. Moreover, proliferative responses to immobilized anti-CD3 were decreased in peripheral Thy-1<sup>−/−</sup> lymphocytes; this decrease was associated with a significantly reduced intracellular Ca<sup>2+</sup> influx and protein tyrosine phosphorylation, indicating impairment of early lymphocyte activation. In contrast, the T cell proliferation induced by mitogens was normal, suggesting that Thy-1 expression weakly contributes to TCR-mediated T cell activation. Epidermal Langerhans cells and bone marrow-derived dendritic cells from Thy-1<sup>−/−</sup> mice exhibited a normal expression of costimulatory surface molecules as well as an unaltered ability to stimulate allogeneic T cells. Taken together, these findings demonstrate that a lack of Thy-1 expression does not generally compromise the immune system; however, Thy-1 expression may be involved in the fine-tuning of T cell-mediated immune responses. The Journal of Immunology, 1998, 161: 5296–5302.

Materials and Methods

Mice

Thy-1-deficient (Thy-1<sup>−/−</sup>) mice were established in a 129Sv × C57BL/6 background as described previously (7). F<sub>1</sub> littermates from the intercross of F<sub>1</sub> Thy<sup>+/−</sup> mice were used for comparative studies between Thy-1<sup>−/−</sup> and Thy-1<sup>+/+</sup> mice.

CHS, DTH, irritant dermatitis, and immunohistochemistry

CHS and DTH experiments were performed as described previously (8, 9). Briefly, mice (n = 5) were sensitized by painting 100 µl of 0.15% trinitrochlorobenzene (TNCB) or 50 µl of 2% oxazolone in acetone/corn oil (4:1) onto the shaved back. For elicitation of CHS responses, 10 µl of 0.8% TNCB or 0.5% oxazolone was painted on both sides of each ear. CHS was determined by the degree of ear swelling of the hapten-exposed ear compared with the ear thickness before challenge and was measured with a micrometer (Mitutoyo, Tokyo, Japan) at 24 and 48 h postchallenge. Mice that were ear challenged without prior sensitization served as negative controls.

Abbreviations used in this paper: CHS, contact hypersensitivity; DTH, delayed-type hypersensitivity; BmDC, bone marrow-derived dendritic cell; GM-CSF, granulocyte-macrophage CSF; MELR, mixed epidermal cell-lymphocyte reaction; DETC, dendritic epidermal T cell; PTK, protein tyrosine kinase; TNCB, trinitrochlorobenzene.
For assessment of DTH responses, nucleated spleen cells from naive BALB/c (H-2d) mice were used. For sensitization, 1 x 10^7 spleen cells were injected s.c. at two sites at the dorsum of naive Thy-1^-/- and Thy-1^+/- mice (n = 5; H-2d). Control groups received equivalent injections of PBS. For elicitation of DTH, mice were challenged by a s.c. injection of 1 x 10^7 spleen cells (suspended in 30 μl of PBS) into one hind footpad. Control groups that had not been sensitized received identical footpad injections. DTH was determined by the degree of footpad swelling of the injected site compared with the untreated contralateral footpad and was measured with a micrometer at 24 h postchallenge.

To assess irritant dermatitis, groups of mice (n = 5) were painted with 10 μl of 1% croton oil in acetone/corn oil (4:1) on both sides of each ear. Ear swelling was measured 24 h later as described above and compared with the ear thickness before painting. Mice painted only with acetone/corn oil served as a negative control.

For immunohistochemical analyses, deparaffinized embedded sections were mounted on Tissue-Tek (Mikron, Walldorf, Germany), washed in 1% methanolic hydrogen peroxide, rinsed with PBS, preincubated with 2% BSA for 30 min, and incubated with the primary Abs rat anti-mouse CD4 (clone H 129.19, Pharmingen, San Diego, CA) and rat anti-mouse CD8a (clone 53-6.7, Pharmingen) diluted 1/10 in 1% BSA for 2 h at room temperature. Sections were developed by an indirect immunoperoxidase technique using the following reagents: PBS (0.01 M, pH 7.4), peroxidase-conjugated goat anti-rat IgG (dilution 1/100, Dianova, Hamburg, Germany), 0.01% hydrogen peroxide, and 3-amino-9-ethylcarbazole (Sigma, St. Louis, MO). Incubation of sections of mouse skin with anti-mouse CD4 and anti-mouse CD8 (for negative controls the unconjugated Ab was used) was followed by a peroxidase-conjugated goat anti-rat IgG Ab (dilution 1/100, Dianova). Counterstaining was performed with Meyer’s hemalaun solution (Merck, Darmstadt, Germany).

**Generation and culture of BmDCs**

BmDCs were generated by the culture of bone marrow cells in the presence of granulocyte-macrophage colony stimulating factor (GM-CSF) and IL-4, as described by Inaba et al. (10). Briefly, bone marrow was collected from tibias and femurs of Thy-1^-/- and Thy-1^+/- mice, using PBS and a syringe with a 25-gauge needle, and suspended by vigorous pipetting. Erythrocytes were lysed by incubating cells in lysing buffer (Ortho, Neckargmünd, Germany) for 2 min. The remaining cells were passed through nylon mesh to remove small pieces of bone and debris. The cells were washed twice with cold PBS, resuspended in BM medium (RPMI 1640 medium supplemented with 5% FCS, 10% heat-inactivated FCS, 100 U/ml penicillin, 100 mg/ml streptomycin, 0.1 mM essential and nonessential amino acids, 2 mM l-glutamine, 1 mM sodium pyruvate, and 0.01 M HEPES buffer (“complete medium”) containing 50 μg/ml mitomycin C (Sigma) for 30 min (37°C, 5% CO_2); washed six times with cold PBS; and applied in serial dilutions to 2 x 10^6 allogeneic T cells in 96-well round-bottom plates. T cells were obtained from the spleen cells of BALB/c mice by nylon wool purification. After 4 days, T cell proliferation was measured by adding 1 μCi of [3H]thymidine per well followed by quantification of incorporated [3H]thymidine.

**Mixed epidermal cell-lymphocyte reaction (MELR)**

Primary MELR was performed as described previously (9, 12). T cells were prepared by passing RBC-depleted spleen cells over a nylon wool column followed by the removal of the remaining contaminants using mAbs M5/114, Mac-1, and B220 with immunomagnetic microbeads (Miltenyi Biotec, Bergisch Gladbach, Germany). The resulting cell preparation contained <0.1% IA^+ cells. T cells were then cocultured with freshly prepared epidermal cells from Thy-1^-/- and Thy-1^+/- mice in complete medium without essential amino acids and FCS and supplemented with 1.5% mouse serum and 5 μg/ml indomethacin ("MELR medium"). Serial dilutions of triplicate samples of epidermal cells were mixed with a constant amount (2 x 10^6) of allogeneic T cells in round-bottom 96-well plates. Cells were cultured for 6 days and pulsed with 1 μCi of [3H]thymidine per well for 18 h. Subsequently, cells were harvested for the evaluation of [3H]thymidine incorporation.

**Lymphocyte proliferation and cytokine quantification**

Nylon wool-enriched splenic T cells were used for proliferation experiments. The stimulation of T cells was performed with either Con A (3 μg/ml) or PMA (3 ng/ml) plus ionomycin (300 ng/ml) (all from Sigma) for 48 h. For anti-CD3 stimulation, 100 μl supernatant from the clone 145-2C11 (American Type Culture Collection, Manassas, VA) was used to coat each well of a 96-well plate at 4°C overnight. T cells were subsequently incubated in these precoated plates for 48 h. T cell proliferation was measured by adding 1 μCi of [3H]thymidine per well followed by quantification of incorporated [3H]thymidine at 16–18 h after pulsing. IL-2 concentrations were measured using a commercially available ELISA (Biosource, Ratigen, Germany).

**Western blotting**

Freshly collected lymph node cells (1 x 10^7) were stimulated with anti-CD3 mAb (145-2C11, 20 μg/ml) at 37°C for various periods of time. Cells were then immediately diluted with 15 ml ice-cold PBS, pelleted, and lysed in lysis buffer (10 mM Tris-Cl (pH 7.5); 150 mM NaCl; 1 mM EDTA; 1 mM sodium pyrophosphate; 1 mM NaF; 1 mM Na_3VO_4; 5 mg/ml each of leupeptin, aprotinin, and pepstatin; and 0.5% Triton X-100). Lysates were centrifuged (12,000 x g, 10 min, 4°C) to remove insoluble material. The proteins were resolved on 12.5% acrylamide gels (Mini-PROTEAN, Bio-Rad, Mississauga, ON) and transferred to a polyvinylidene difluoride membrane that was then incubated with an anti-phosphotyrosine material. The proteins were resolved on 12.5% SDS-PAGE and transferred to a polyvinylidene difluoride membrane that was then incubated with an anti-phosphotyrosine mAb (4G10, Santa Cruz Biotechnology, Santa Cruz, CA). Equal loading of each lane was determined using an anti-Lck mAb (21C2, Santa Cruz Biotechnology). Staining of the anti-phosphotyrosine mAb and anti-Lck mAb was performed using an anti-mouse Ig coupled to a horseradish peroxidase enhanced chemiluminescence system (Amersham, Freiburg, Germany).

**Calcium analysis**

Lymph node cells were initially loaded with Indo-1 (Sigma) before labeling with Na_2F-specific anti-CD4 and anti-CD8 mAb. After prewarming to 37°C, purified anti-CD3 mAb (40 μg/ml) was added to the cell samples (at 2 min of recording); Ca^{2+} influx in T lymphocytes was measured on a FACStar Plus (Becton Dickinson). Data were analyzed using MTIME software (Phoenix Flow Systems, San Diego, CA).

**Statistics**

A total of five mice were used per group in each CHS or DTH experiment. All experiments were performed at least three times. The data presented show the values obtained from one representative experiment. Data were analyzed using the Student’s t test for independent samples.

**Results**

**Decreased CHS responses in Thy-1-deficient mice**

To study the effects of Thy-1 deficiency in CHS responses to contact allergens, age-matched Thy-1^-/- and Thy-1^+/+ control mice
were sensitized to oxazolone. At 5 days after sensitization, these groups and their nonsensitized controls were challenged on the ears; CHS responses were assessed 24 h later. The data depicted in Fig. 1A demonstrate a significantly decreased swelling response of Thy-1<sup>−/−</sup> mice compared with Thy-1<sup>+/+</sup> controls. An additional experiment was also performed using TNCB as a contact allergen. Again, a significantly suppressed CHS response was seen after TNCB challenge in sensitized Thy-1<sup>−/−</sup> mice compared with sensitized Thy-1<sup>+/+</sup> controls (data not shown). In addition, histologic evaluation of the CHS reactions revealed a significant reduction in the inflammatory cellular infiltrate and edematous tissue swelling in Thy-1<sup>−/−</sup> mice as opposed to Thy-1<sup>+/+</sup> controls (Fig. 1, B and C), which suggests a role for Thy-1 in murine CHS reactions.

Immunohistochemical analyses were performed to evaluate the CD4/CD8 ratio of the lymphocyte infiltrate in these lesions. No differences in the subset ratio in Thy-1<sup>−/−</sup> mice compared with Thy-1<sup>+/+</sup> controls were detected (data not shown).

Moreover, the irritant dermatitis induced by topical application of 1% croton oil was also found to be significantly decreased in Thy-1-deficient mice compared with Thy-1<sup>+/+</sup> controls, as depicted in Fig. 1D. To exclude the possibility that altered numbers of Langerhans cells and dendritic epidermal T cells (DETCs) were responsible for this effect, we determined the number of Langerhans cells and DETCs present in the epidermal sheets of Thy-1<sup>−/−</sup> and Thy-1<sup>+/+</sup> mice. Thy-1-deficient mice were found to have normal numbers of

FIGURE 1. A, Decreased hapten-specific CHS responses in Thy-1-deficient mice. Thy-1<sup>−/−</sup> and Thy-1<sup>+/+</sup> mice (n = 5 per group) were sensitized with 2% oxazolone on the shaved back. After 5 days, mice were challenged with 0.5% oxazolone on both sides of each ear; after 24 h, ear swelling was measured and compared with the ear thickness determined before challenge. Control mice were not sensitized and were ear challenged only. Data were obtained from one representative experiment of three and show mean ear swelling ± SEM. p values are as follows: p < 0.001 for Thy-1<sup>+/+</sup> vs Thy-1<sup>−/−</sup>, Thy-1<sup>−/−</sup> only challenged, and Thy-1<sup>+/+</sup> only challenged. B, Histopathology of Thy-1<sup>+/+</sup> CHS lesions (hematoxylin/eosin (HE), ×80). Dense lymphohistiocytic inflammatory infiltrate with some neutrophils and edema within the dermal tissue of the ear of oxazolone-sensitized and -challenged mice was observed. C, Histopathology of Thy-1<sup>−/−</sup> CHS lesions (HE, ×80). Less cellular inflammatory infiltrate and less edema in the dermal tissue of the ear of oxazolone-sensitized and -challenged mice was seen. D, Decreased irritant dermatitis in Thy-1-deficient mice. Thy-1<sup>−/−</sup> and Thy-1<sup>+/+</sup> mice (n = 5 per group) were painted with 10 μl of 1% croton oil on both sides of each ear. After 24 h, ear swelling was assessed as a measure of irritant dermatitis and compared with ear thickness before painting. Data are from one representative experiment of three and show mean ear swelling after 24 h ± SEM. p values are as follows: p < 0.004 for Thy-1<sup>+/+</sup> vs Thy-1<sup>−/−</sup>.
DETCs and Langerhans cells as determined by the staining of epidermal sheets with a fluorescence-labeled anti-CD3 mAb or anti-I-Ab mAb (data not shown).

Decreased DTH to alloantigens in Thy-1-deficient mice

To address the question of whether Thy-1 plays a role in DTH reactions to alloantigens, Thy-1\(^{+/+}\) and Thy-1\(^{-/-}\) control mice (both H-2\(b\)) were immunized to alloantigens by s.c. injection of nucleated H-2\(^d\) spleen cells (BALB/c). After 5 days, these groups and nonsensitized groups of Thy-1\(^{-/-}\) and Thy-1\(^{+/+}\) mice (n = 5 per group) were challenged with H-2\(^d\) spleen cells in one hind footpad; 24 h later, footpad swelling was determined as the difference in swelling between the injected and noninjected sides. Data are from one representative experiment of three and show mean footpad swelling after 24 h ± SEM. p values: p < 0.03 for Thy-1\(^{-/-}\) vs Thy-1\(^{-/-}\) and Thy-1\(^{+/+}\) only challenged.

### Figure 2

**Reduced DTH responses to alloantigens in Thy-1-deficient mice.** Thy-1\(^{-/-}\) and Thy-1\(^{+/+}\) mice (H-2\(b\)) were sensitized to alloantigens by s.c. injection of nucleated H-2\(^d\) spleen cells (BALB/c). After 5 days, these groups and nonsensitized groups of Thy-1\(^{-/-}\) and Thy-1\(^{+/+}\) mice (n = 5 per group) were challenged with H-2\(^d\) spleen cells in one hind footpad; 24 h later, footpad swelling was determined as the difference in swelling between the injected and noninjected sides. Data are from one representative experiment of three and show mean footpad swelling after 24 h ± SEM. p values: p < 0.03 for Thy-1\(^{-/-}\) vs Thy-1\(^{-/-}\) and Thy-1\(^{+/+}\) only challenged.

Stimulation of allogeneic T cells by BmDCs or epidermal cells of Thy-1-deficient mice

To test whether an impaired ability to present Ag could account for the reduced CHS and DTH reactions in Thy-1-deficient mice, BmDCs were prepared from Thy-1\(^{-/-}\) and Thy-1\(^{+/+}\) control mice and used to stimulate allogeneic T cells in the mixed BmDC-lymphocyte reaction. The data in Fig. 3A demonstrate a similar stimulation of allogeneic T cells by various concentrations of BmDCs from Thy-1\(^{-/-}\) mice compared with Thy-1\(^{+/+}\) control mice. Furthermore, a portion of these BmDCs was evaluated for various cell surface markers by flow cytometry. Neither I-A\(^b\), CD40, nonlymphoid dendritic cell 145, nor CD80 or CD86 surface marker expression was markedly altered in Thy-1\(^{-/-}\) mice compared with Thy-1\(^{+/+}\) control mice (data not shown). In addition, epidermal cells from Thy-1\(^{-/-}\) mice and Thy-1\(^{+/+}\) mice were able to stimulate allogeneic T cells to a magnitude similar to that of Thy-1\(^{+/+}\) control cells (Fig. 3B). This finding suggests that BmDCs as well as epidermal cells from Thy-1\(^{-/-}\) mice are able to stimulate allogeneic T cells equally as well as Thy-1\(^{+/+}\) controls, indicating that the ability to present Ags is not impaired in Thy-1-deficient mice.
Impaired tyrosine phosphorylation and calcium influx after anti-CD3 stimulation in peripheral T cells of Thy-1−/− mice

As Thy-1 has been shown to regulate TCR signaling in thymocytes, we analyzed early TCR-mediated signaling events in peripheral T lymphocytes from Thy-1+/+ and Thy-1−/− mice. The earliest detectable effects that follow TCR engagement are a series of tyrosine phosphorylation events initiated by activated protein tyrosine kinases (PTKs), which are essential in coupling the TCR to the downstream signaling pathways. These initial events include hydrolysis of inositol-containing phospholipids, Ca2+ mobilization, and activation of the Ras/mitogen-activated protein kinase cascade. The data presented in Fig. 4 show the kinetics of tyrosine phosphorylation of substrates in Thy-1−/− and Thy-1+/+ lymphocytes after TCR/CD3 cross-linking. T cells were stimulated at various timepoints with an anti-CD3 mAb and lysed immediately. Tyrosine-phosphorylated proteins were analyzed by immunoblotting with an Ab directed against phosphorytrosine. Increases of tyrosine phosphoproteins in Thy-1−/− mice were maximally detected following a 2–5 min stimulation. Although a similar kinetic of tyrosine phosphorylation of substrates following anti-CD3 mAb cross-linking was observed in T cells from Thy-1−/− mice, there was a significant decrease in the overall intensity of tyrosine phosphoproteins. This finding suggests an impairment in Thy-1−/− peripheral T lymphocytes in the TCR-triggered activation of PTKs. This impairment is consistent with an alteration in the downstream signaling event. The data in Fig. 5 demonstrate that the calcium mobilization in Thy-1−/− T cells by anti-CD3 mAb cross-linking was significantly reduced compared with their Thy-1+/+ counterparts. In another experiment, Thy-1−/− and Thy-1+/+ T cells demonstrated identically increased intracellular Ca2+ stores by blocking the endoplasmic reticulum ATPase/Ca2+ pump (data not shown).

T cell proliferation and cytokine secretion in Thy-1−/− deficient mice

Taking into account the impaired Ca2+ influx and the reduced tyrosine phosphorylation in Thy-1−/− deficient T cells, we were interested in investigating the proliferative response and cytokine expression of Thy-1−/− peripheral T cells after various stimuli. Thy-1−/− deficient mice had similar numbers and subsets of peripheral T lymphocytes as Thy-1+/+ mice. Nylon wool-enriched splenic T cells were stimulated with either immobilized anti-CD3, Con A, or PMA plus ionomycin. Subsequently, proliferation was measured by assessing the radio-labeled thymidine uptake. The data in Table I show that treatment with immobilized anti-CD3, but not stimulation of Thy-1−/− T cells with Con A or PMA plus ionomycin, resulted in altered proliferative responses in Thy-1−/− T cells compared with Thy-1+/+ control T cells. Subsequently, culture supernatants were harvested and assayed for the presence of IL-2, IL-4, and IFN-γ. The data in Table II indicate that the production of IL-2, IL-4, and IFN-γ after various stimuli is similar in Thy-1−/− T cells and Thy-1+/+ control T cells. These data suggest that Thy-1 expression may play a role during proliferative T cell responses after TCR/CD3 stimulation, and that Thy-1 deficiency might not be able to perturb the cytokine secretion pattern of these cells.

Table I. Thy-1−/− T lymphocyte activation after stimulation

<table>
<thead>
<tr>
<th>Stimulusa</th>
<th>Thy-1−/−</th>
<th>Thy-1+/+</th>
</tr>
</thead>
<tbody>
<tr>
<td>ϕ</td>
<td>821 ± 92</td>
<td>811 ± 220</td>
</tr>
<tr>
<td>Anti-CD3</td>
<td>91.960 ± 9328</td>
<td>120.495 ± 2783b</td>
</tr>
<tr>
<td>Con A</td>
<td>238.315 ± 8567</td>
<td>242.975 ± 4692</td>
</tr>
<tr>
<td>PMA and ionomycin</td>
<td>280.635 ± 5695</td>
<td>260.035 ± 7889</td>
</tr>
</tbody>
</table>

a T lymphocytes (2 × 10⁶) were stimulated for 48 h as described previously. 
[¹H]thymidine was added, and incorporation was measured (cpm ± SEM). 

b p < 0.04 for Thy-1−/− vs Thy-1+/+. Data represent one of three independent experiments.

ϕ No stimulation.
Table II. Lymphokine production by Thy-1\(^{-/-}\) and Thy-1\(^{+/+}\) lymphocytes after stimulation\(^a\)

<table>
<thead>
<tr>
<th>Stimulus</th>
<th>IL-2 (U/ml)</th>
<th>IL-4 (U/ml)</th>
<th>IFN-(\gamma) (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C2b</td>
<td>Thy-1(^{-/-})</td>
<td>&lt;0.1</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td></td>
<td>Thy-1(^{-/-})</td>
<td>&lt;0.1</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>Anti-CD3</td>
<td>Thy-1(^{-/-})</td>
<td>22</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Thy-1(^{+/+})</td>
<td>25</td>
<td>6</td>
</tr>
<tr>
<td>Con A</td>
<td>Thy-1(^{-/-})</td>
<td>18</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>Thy-1(^{+/+})</td>
<td>14</td>
<td>7</td>
</tr>
<tr>
<td>PMA and ionomycin</td>
<td>Thy-1(^{-/-})</td>
<td>&gt;50</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td>Thy-1(^{-/-})</td>
<td>&gt;50</td>
<td>28</td>
</tr>
</tbody>
</table>

\(^a\) T lymphocytes (2 \times 10^5) were stimulated for 48 h as indicated. Culture supernatants were then harvested and assayed for lymphokine content as described previously.

Discussion

Although the structure of Thy-1 is well understood, comparatively little is known about the in vivo role of Thy-1 in immune responses. Thy-1-deficient mice are healthy and show no signs of obvious immunodeficiency (7). Thymocytes, which normally express high amounts of Thy-1 in wild-type mice, exhibit impaired maturation from the CD4\(^+/\)CD8\(^-\) double-positive stage to the single-positive stage in Thy-1\(^{-/-}\) mice, possibly due to inappropriate negative selection (6). The abnormal development of Thy-1\(^{-/-}\) thymocytes is accompanied by a hyperresponsiveness to TCR stimulation. In vitro cross-linking of TCR/CD3 did induce heightened immunoreceptor tyrosine-based activation motif phosphorylation in both p23 TCR and CD3\(\varepsilon\) chains, as mediated by the Lck srk kinase. This alteration in early TCR signaling seems to be responsible for enhanced downstream signaling events such as Ca\(^{2+}\) mobilization and cell proliferation. Consistent with enhanced negative selection, a recent report using Thy-1\(^{-/-}\) mice containing a TCR transgene specific for a MHC class II-restricted Ag indicated that normal self-tolerance occurs in the absence of Thy-1 (15).

In the present study, Thy-1\(^{-/-}\) mice demonstrated reduced cutaneous immune responses compared with Thy-1\(^{+/+}\) mice but were not completely unable to develop CHS or DTH reactions. After CD3/TCR stimulation, Thy-1\(^{-/-}\) peripheral T cells showed decreased proliferative responses and less influx of free Ca\(^{2+}\) into the cytoplasm. In addition, CD3/TCR activation led to less protein tyrosine phosphorylation in Thy-1\(^{-/-}\) T cells than in Thy-1\(^{+/+}\) controls, suggesting that Thy-1 is one modulator of T cell function. Our findings indicate that Thy-1 is not essentially required for the complex signaling events that orchestrate cutaneous immune responses but appears to be involved in the fine-tuning of T cell function. Since Thy-1 is absent on all lymphocytes of these deficient mice, both Ag-specific and Ag-nonspecific cutaneous inflammation models are involved. Thus, impaired T cell activation could be an explanation for decreased T cell-mediated immune responses in Thy-1-deficient mice.

There is also evidence for a role of Thy-1 in the activation of peripheral T cells. The physiologic ligand of Thy-1 is still unknown, but ligation of Thy-1 by several anti-Thy-1 mAbs reacting with different epitopes on the molecule results in the proliferation of various T cell clones and T cell hybridomas (2, 16, 17). This proliferative response occurs in parallel with a rise in free intracellular Ca\(^{2+}\). Our own data support these findings, since peripheral T cells from Thy-1-deficient mice demonstrated less proliferative responses and less free intracellular Ca\(^{2+}\) after CD3/TCR activation. Other reports indicated that in Thy-1\(^{-/-}\) and CD3/TCR\(^{-/-}\) variants of T cell clones, the cotransfection of Thy-1 and CD3/TCR complexes was needed to observe IL-2 secretion as a measure of proliferation after Thy-1 stimulation by anti-Thy-1 Ab (1). Moreover, T cell activation induced by glycosphatidylinositol-linked proteins is closely linked to the production of IL-2 as a late event of stimulation that favors autocrine growth (3). Our findings demonstrate that Thy-1-deficient lymphocytes are able to produce similar amounts of IL-2, IL-4, or IFN-\(\gamma\) after CD3/TCR activation, indicating that Thy-1 may not be involved in the production of IL-2.

Signal transduction through the TCR or through glycosphatidylinositol-linked cell surface molecules such as Thy-1 appears to activate cytoplasmic PTKs, although no clear intrinsic PTK activity has been demonstrated by the TCR or Thy-1. Lck and Fyn, which are members of the Src family of PTKs, seem to be critically involved in TCR- and Thy-1-induced signaling (18). In particular, cellular Fyn has been shown to be associated with Thy-1 as detected by coimmunoprecipitation. Accordingly, mice that underwent gene-targeted disruption of the lymphocytic form of Fyn showed profound functional defects in mature thymocytes after Thy-1 activation in terms of intracellular Ca\(^{2+}\) elevation, IL-2 production, and proliferation (19). Our findings further support a role for Thy-1 in protein tyrosine phosphorylation, because T lymphocytes from Thy-1-deficient mice demonstrated less tyrosine phosphorylation after TCR/CD3 stimulation. This decrease in tyrosine phosphorylation in Thy-1\(^{-/-}\) lymphocytes was found to be particularly associated with bands of 21, 36, 50, and 68 kDa. The p21 and p36 could represent the phosphorylated TCR\(\zeta\) and LAT, respectively, as they are known to be the major tyrosine-phosphorylated bands in this molecular mass range (20). Moreover, their phosphorylation is known to link the TCR to downstream signaling events such as calcium mobilization.

Alternatively, the impaired peripheral T lymphocyte activation in Thy-1\(^{-/-}\) mice could be a consequence of the thymic developmental defects found in these animals. In fact, it is possible that, in the absence of the negative regulation of TCR signaling in thymocytes by Thy-1, an inappropriate negative selection process leads to the accumulation of thymocytes that normally display a reduced signaling capability via TCR. Once they have emigrated to the periphery, these T cells will suboptimally respond to TCR signaling.

In addition to being involved in lymphocyte activation, murine Thy-1 has also been shown to be involved in cell to cell adhesion, notably in the binding of murine thymocytes to mouse thymic epithelial cells (4). In this system, thymoepithelial interactions involved the binding of Thy-1 to sulfated glycans such as pentosan sulfate, dextran sulfate, and fucoidan, which were inhibited by \(\leq 40\%\) by soluble Thy-1 molecules and did not require Ca\(^{2+}\) (21). Therefore, it is possible that cell to cell interactions between lymphocytes and epithelial cells or APCs could be altered in Thy-1\(^{-/-}\) mice. Such a putative, defective interaction could consequently be an alternative explanation for the decreased CHS and DTH responses in Thy-1-deficient mice. However, we do not have any evidence for such a mechanism at this time, although current studies are underway to further test this hypothesis.

Finally, one could speculate that Thy-1\(^{-/-}\) DETCs may also be involved in differences in cutaneous immune responses between Thy-1\(^{-/-}\) and Thy-1\(^{+/-}\) mice. Murine skin also contains a population of DETCs, which have been identified by their expression of Thy-1 (22, 23). DETCs are bone marrow-derived T cells that express the TCR \(\gamma\)- and \(\delta\)-chains (24). Apart from being a marker for these cells, there is no evidence that Thy-1 expression is of particular functional relevance in these cells. Studies with a recently generated murine Thy-1\(^{-/-}\)-DETC line indicate that these cells may...
participate in the modulation of the skin immune system by secretion of a distinct pattern of cytokines (25). However, Thy-1-deficient mice were found to have normal numbers of DETCs, as visualized by staining for CD3. Thus, it is unlikely that differences in the number of DETCs account for the impaired immunity in vivo.

Taken together, our data show that Thy-1 deficiency leads to an impairment of in vivo T cell-mediated immune responses. This impairment might be due to a defective fine-tuning of T cell effector functions in the absence of Thy-1.

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References