Induction of Tolerance to Autoimmune Diabetes with Islet Antigens

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Summary

The development of T cell tolerance to self-antigens is imparted principally through negative selection events during thymic ontogeny. However, this tolerance may be limited to antigens that are expressed in the thymus, and additional mechanisms are probably required to regulate autoimmune responses to tissue-specific antigens. Autoimmune diabetes can be induced experimentally by treating susceptible strains of mice with multiple low doses of streptozotocin (STZ). In this report we show that transplantation of isolated islets of Langerhans into the thymuses of adult C57BL/KsJ mice will induce tolerance to the subsequent induction of autoimmune diabetes. This tolerance is tissue specific and thymus dependent. It was not induced by thymic transfer of adrenal tissue or by kidney transfer of islets. Furthermore, depletion of mature T cells was required and the tolerant state was abrogated by the adoptive transfer of normal splenocytes.

It is interesting that pretreatment of the islets with STZ enhanced their ability to induce tolerance, and suggests that antigen shedding induced by tissue damage may facilitate transfer of islet antigens to tolerizing cells in the thymus. These findings indicate that thymic tolerance specific for tissue can be stimulated to occur in the presence of atopical tissue-specific intrathymic antigens. Elimination of disease-related T cells in the absence of global immunosuppression represents a novel approach for the prevention of autoimmune disease.

Antigens encountered by T cell precursors during maturation in the thymus shape the repertoire of peripheral T cells. Initially, cells capable of reacting with antigens presented in the context of self MHC antigens develop through a process of thymic learning termed positive selection (1). Those T cells with the potential for autoreactivity are eliminated during passage into the thymic medulla. This event, termed negative selection or clonal deletion, is thought to prevent the escape of autoreactive T cells into the peripheral tissues and represents the major mechanism for tolerance to self-antigens (2-4). Negative selection has been shown for T cells reactive with antigens expressed in the thymus such as I-E (5) and Mls (6), or H-Y antigen (7). Although it is an effective mechanism for deletion of cells reactive with antigens that are encountered in the thymus, clonal deletion may not always be complete, especially for antigens that are found only in the periphery (8-10). Thus, the repertoire of circulating T cells includes cells that are capable of responding to antigens expressed exclusively in the peripheral tissues (8, 9). These cells may be capable of causing autoimmune disease with appropriate activation signals and antigen presentation (9).

The multi-dose streptozotocin model (MDSM) of insulin-dependent diabetes mellitus (IDDM) is a T cell-mediated autoimmune disease (11). In this model, hyperglycemia and insulitis are induced about 10 d after administration of five subdiabetogenic doses of streptozotocin (STZ) in certain strains of male mice. Both insulitis and hyperglycemia can be prevented by T cell depletion using mAbs (12), and adoptively transferred with splenocytes (13). The mechanism of the disease and the effects of STZ on the islet cells are incompletely understood, but recent studies by Weide and Lacy (14) have shown that STZ treatment of islets is required to render them antigenic. These results suggest that a specific antigen(s), as yet unknown, is expressed by damaged islets which triggers autoreactive T cells.

Posselt et al. (15) have described a system whereby tolerance to alloantigen can be induced by surgical implantation of allogenic islet cells into the thymus. Their study suggests that if T cells mature in the thymus in the presence of alloantigen...
tigen, deletion or inactivation of alloreactive cells that norma-

ly populate the periphery can be induced. However, this

study with alloantigen, as well as those previously reported

involving Mls (6), IE (5), or H-Y (7), involve antigens that

are not tissue specific and for which there is a relatively high

frequency of antigen-reactive T cell precursors. It is unknown

whether in adult animals, thymic tolerance can be induced

towards tissue-specific antigens and whether it can be used to

prevent organ-specific disease. We have, therefore, deter-
mind whether thymic transplantation of islet cells that ex-

press antigens that are recognized in autoimmune diabetes
can induce tissue-specific tolerance in a manner that will protect

gainst the development of disease.

MATERIALS AND METHODS

Animals. Male C57BL/KsJ mice were purchased from The

Jackson Laboratory (Bar Harbor, ME) and used between the ages

of 5 and 9 wk. The mice were housed under pathogen-free con-
tions in the animal barrier facility at The University of Chicago.
The animals were allowed access to standard laboratory chow and

water ad libitum.

Preparation of Animals. Islets of Langerhans were isolated by

standard collagenase technique (16). The islets were handpicked

from the digest of pancreas with the aid of a stereomicroscope

and placed in culture overnight with DMEM (containing 25 mM

glucose) with 10% FCS, 10 mM morpholino propane sulfonic acid

(MOPS), penicillin (100 U/ml), streptomycin (100 /g/ml), and

adicion amino acids. The islets were again harvested by hand with

a stereomicroscope and washed three times. They were treated for

30 min in Krebs-Hepes buffer (Hanks' buffered saline with 10

mM Hepes, 1% BSA, 14 mM sodium bicarbonate) with STZ (0.5

mg/ml), washed, and suspended in Krebs-Hepes buffer. Between

100 and 125 islets (in 25 pl) were injected into each of two lobes

of the thymus of adult C57BL/KsJ mice. Some animals received

an equivalent number of islets that were not treated with STZ,
or minced fragments of adrenal tissue, that were (n = 2), or were

not (n = 2) treated with STZ for 30 min as described for islets.

The adrenal tissue was taken from islet donors. In other mice,

200-250 islets were transplanted under a kidney capsule. On the
day after surgery, each of the mice was treated with anti-CD3 mAb

(145-2C11, 300 /g intraperitonially, purified with protein

A-Sepharose from asites). A fourth group of mice received in-

trathymic STZ-treated islets, but did not receive anti-CD3 mAb


treatment.

Induction of MDSDM. 3.5 wk after surgery and treatment with

anti-CD3 mAb, the mice were given STZ (40 mg/kg, in citrate

buffer, intraperitonially) daily for 5 d, to induce diabetes (11, 12).

This protocol causes insulitis and hyperglycemia in ~75-80% of

normal C57BL/KsJ mice 1-2 wk after the final dose of STZ (12).

Glucose levels were measured using a glucose analyzer (Beckman

Instruments, Inc., Berkeley, CA) in plasma obtained from the ret-
orbital sinus on days 12 and 16, corresponding to the 7th and 11th
days after the fifth injection of STZ. In one experimental group,

STZ-treated islets were placed into the thymus followed by anti-

CD3 mAb as described, but the mice were administered 40 x 10^6

splenocytes freshly isolated from another normal male C57BL/KsJ

mouse 3 h before the first of five injections of STZ. The differences

between the mean plasma glucose levels of each of the groups were

compared by a Student's t test. Differences in the incidence of dia-

betes between the treatment and control groups were analyzed by

Fisher's exact test.

Analysis of Splenocytes by Flow Cytometry. Single cell suspensions

of splenocytes were prepared from mice receiving the indi-
cated treatment and stained first with mAb 2.4G2 (17) (antimu-

rine Fc receptor) followed by mAbs against CD3 (mAb 145-2C11;

Boehringer Mannheim Diagnostics, Inc., Indianapolis, IN), CD4

(Boehringer Mannheim Diagnostics, Inc.), CD8, and Thy1.2

(Becton Dickinson & Co., Mountainview, CA), which were directly

coujugated to FITC. The stained cells were analyzed on a FACScan*

(Becton Dickinson & Co.) with electronic gates placed around the

peak corresponding to hemocytometry. Data from 10,000 cells were

collected. The percent positive cells represent the percentage of cells

with fluorescence intensity above staining with a FITC-conjugated

irrelevant mAb (anti-Leu4, Becton Dickinson & Co.). The data

presented in Table 1 were collected from five to seven mice in each

group and represent mean _+ SEM. The mean values in the experi-

mental groups were compared with control values by a Stu-

dent's t test.

Histologic Analysis of Insulitis. The pancreas from animals

receiving the indicated treatments (14 from the group receiving

intrathymic injection of buffer and anti-CD3 mAb and 7 from the

group receiving intrathymic STZ-treated islets and anti-CD3 mAb)

were harvested on days 16-18 and placed in formalin solution. Sec-

tions of the fixed pancreases, embedded in paraflin were prepared

and stained with hematoxylin and eosin. Without knowledge of

the source of the section, the presence of insulitis was evaluated

by one of us (A. Montag), and graded as follows: 0, normal

histology; 1, minimal cellular infiltrate into the islets, otherwise

normal islet architecture; 2, extensive cellular infiltrate but preser-

vation of islet architecture; 3, cellular infiltrate and loss of normal

islet architecture. The differences in the scores between the two

groups were compared by /-test.

Immunohistochemical Staining for Insulin. At the time of killing,

12 d after the last dose of STZ, the thyms and pancreas from

animals were fixed in Bouin's fixative, and stained with guinea pig

antinsulin antibody using the avidin-biotin peroxidase method (18).

Tissue sections were incubated with guinea pig antiinsulin anti-

body (Dako Corp., Carpeteria, CA) washed, and incubated with

biotinylated goat anti-guinea pig antibody (Vector Laboratories,

Inc., Burlingame, CA) followed by streptavidin complex (Dako

Corp.). After washing, antibody complexes on the sections were

localized with dianinobenzidene and counterstained with hema-

toxyl.

Results

Prevention of MDSDM after Intrathymic Placement of Islet Antigen, T Cell Depletion and Regeneration. Islets isolated from

normal male (C57BL/KsJ) mice and treated with STZ in vitro,

were placed into the thymses of syngeneic adult C57BL/KsJ

mice. Control mice received an intrathymic injection of Krebs-

Hepes buffer. All mice then were given an injection of anti-

TCR mAb. This treatment caused depletion of about 70%

of T cells in the spleen by 72-96 h, modulation of the TCR on

residual Thy1.2+ cells (Table 1), and elimination of virtually all T cells in the peripheral circulation. In previously

reported studies, the residual T cells in spleen and lymph node

have been found to have profound functional impairments

(19, 20).

We rested the animals for 3.5 wk to allow for regeneration

of T cells, and then analyzed whether the presence of

islet antigens in the thymus modulated the course of the au-
Table 1. Quantitative Analyses of Splenocytes by Flow Cytometry

<table>
<thead>
<tr>
<th>Group</th>
<th>CD3†</th>
<th>CD4†</th>
<th>CD8†</th>
<th>Thy1.2†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intrathymic buffer/anti-CD3 mAb, studied 72-96 h after anti-CD3 mAb</td>
<td>0.8 ± 0.2*</td>
<td>5.9 ± 1.7*</td>
<td>2.7 ± 0.6*</td>
<td>8.6 ± 2.5*</td>
</tr>
<tr>
<td>Animals studied on days 11-13 after STZ:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intrathymic buffer/anti-CD3 mAb</td>
<td>13.4 ± 1.8*</td>
<td>9.6 ± 1.6*</td>
<td>3.6 ± 0.5*</td>
<td>15.4 ± 2.3*</td>
</tr>
<tr>
<td>Intrathymic STZ-treated islets/anti-CD3 mAb</td>
<td>13.4 ± 1.1*</td>
<td>10.1 ± 0.8*</td>
<td>3.4 ± 0.3*</td>
<td>17.9 ± 1.1*</td>
</tr>
<tr>
<td>Untreated control mice</td>
<td>26.2 ± 1.0</td>
<td>16.0 ± 0.3</td>
<td>9.3 ± 0.6</td>
<td>30.2 ± 1.9</td>
</tr>
</tbody>
</table>

*Splenocytes were harvested from groups of five to seven mice at the indicated times and stained with mAbs as described. The percent positive cells was calculated by comparing the staining to an irrelevant FITC-conjugated mAb (anti-human CD3).

*p <0.001 vs control
† Mean ± SEM % of 10,000 gated cells.
§ Percent control.

Table 2. Prevention of Diabetes by Intrathymic Injection of STZ-treated Islets

<table>
<thead>
<tr>
<th>Group</th>
<th>Plasma glucose (mg/dl, mean ± SEM)</th>
<th>No. with diabetes*/Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 12</td>
<td>Day 16</td>
</tr>
<tr>
<td>Intrathymic buffer/anti-CD3 mAb</td>
<td>269 ± 22</td>
<td>316 ± 25</td>
</tr>
<tr>
<td>Intrathymic STZ-treated islets/anti-CD3 mAb</td>
<td>191 ± 14†</td>
<td>212 ± 15‡</td>
</tr>
</tbody>
</table>

* Diabetes was considered present if plasma glucose >240 mg/dl.
† p = 0.011, § p <0.01, and ¶ p <0.001 vs. intrathymic saline and anti-CD3 mAb.
Figure 1. Effects of thymectomy on glucose levels, and adoptive transfer of diabetogenic cells with splenocytes from normal mice. Plasma glucose levels are shown from two mice that received intrathythic STZ-treated islets and anti-CD3 mAb that were studied further. These data are from a single experiment representative of four separate experiments. Both animals received five subdiabetogenic doses of STZ beginning on day 0, and both underwent thymectomy at day 16 (A). One animal received an injection (intraperitoneal) of 40 and 80 x 10^6 splenocytes from normal male C57BL/KsJ mice (●—●) or saline (○—○) on days 22 and 31 (B and D). Both animals received a single injection (intraperitoneal) of STZ (40 mg/kg in citrate buffer) on days 27 and 34 (C and E). Progressive hyperglycemia was seen in the recipient of normal spleen cells.

The absence of hyperglycemia in mice receiving intrathymic STZ-treated islets and anti-CD3 mAb correlated with a decrease in the autoimmune response as indicated by a reduction in cellular infiltrates into the islets (Table 3, Fig. 3). Histologic sections of pancreases from mice given either intrathythic Hepes buffer and anti-CD3 mAb or to untreated control mice (data not shown).

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The presence of insulin-containing cells with immunohistochemical techniques. Single cells and small clumps of insulin containing cells could be identified at the cortical-medullary junction of the thymus (arrows). ×200.

Figure 2. Identification of insulin containing cells in thymus removed from a tolerized mouse. A thymectomy was performed on mice that had received intrathythic STZ-treated islets and anti-CD3 mAb and had not developed diabetes after STZ. The thymus was studied for the presence of insulin-containing cells with immunohistochemical techniques. Single cells and small clumps of insulin containing cells could be identified at the cortical-medullary junction of the thymus (arrows). ×200.
Table 3. Effects of Treatment on the Development of Insulitis

<table>
<thead>
<tr>
<th>Group</th>
<th>Grade of insulitis*</th>
<th>Total no. islets</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intrathymic Krebs-Hepes buffer and anti-CD3 mAb (n = 14)</td>
<td>50 (39%) 42 (33%) 26 (20%) 10 (8%)</td>
<td>128</td>
</tr>
<tr>
<td>Intrathymic STZ-treated islets and anti-CD3 mAb (n = 7)</td>
<td>46 (77%) 14 (23%) 4 (7%) 0</td>
<td>64</td>
</tr>
</tbody>
</table>

Pancreases were fixed in formalin, and sections of tissue (two from each mouse) were stained with hematoxylin and eosin and graded for the presence of insulitis as follows: 0, Normal histology; 1, minimal cellular infiltrate into the islets, otherwise normal islet architecture; 2, extensive cellular infiltrate but preservation of islet architecture; and 3, cellular infiltrate and loss of normal islet architecture. The differences in the scores between the two groups were compared by \( \chi^2 \).

* Insulitis was absent from the islets of untreated normal male C57BL/KsJ mice.

\[ p < 0.0001 \] vs intrathymic Krebs-Hepes buffer and anti-CD3 mAb. The data in this group includes one animal with exocrine pancreatitis. The distribution of lesions without that animal was: 0, 79%, 1, 21%, 2, 0%, and 3, 0%.

against the induction of MDSDM (Table 4). It is interesting that pretreatment of islets with STZ was necessary to render islets effective tolerogens. There was an initial reduction in the plasma glucose levels and incidence of diabetes (\( p < 0.05 \)) 12 d after STZ treatment in mice receiving intrathymic injection of islets that were not treated in vitro with STZ. However, by day 16, diabetes had developed in 80% of the animals, and the blood glucose levels were similar to mice that had received intrathymic injection of Krebs-Hepes buffer (Table 4).

In addition, T cell depletion with anti-CD3 mAb (Table 4), was needed for tolerance to occur. When mice received intrathymic STZ-treated islets but did not receive anti-CD3...
Table 4. Protection from MDSDM Requires that STZ-treated Islets be Present in the Thymus during T Cell Development

<table>
<thead>
<tr>
<th>Group</th>
<th>Plasma glucose (mg/dl, mean + SEM)</th>
<th>No. with diabetes*/Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 12</td>
<td>Day 16</td>
</tr>
<tr>
<td>Intrathymic adrenal fragments/anti-CD3 mAb</td>
<td>275 ± 29</td>
<td>368 ± 11</td>
</tr>
<tr>
<td>Intrarenal STZ-treated islets/anti-CD3 mAb</td>
<td>280 ± 47</td>
<td>303 ± 41</td>
</tr>
<tr>
<td>Intrathymic non-STZ-treated islets/anti-CD3 mAb</td>
<td>224 ± 25</td>
<td>314 ± 40</td>
</tr>
<tr>
<td>Intrathymic STZ-treated islets/no anti-CD3 mAb</td>
<td>325 ± 66</td>
<td>278 ± 37</td>
</tr>
<tr>
<td>Intrathymic STZ-treated islets/anti-CD3 mAb, and syngeneic spleen cells before five doses of STZ</td>
<td>272 ± 46</td>
<td>349 ± 36</td>
</tr>
</tbody>
</table>

* Diabetes was considered present if plasma glucose >240 mg/dl.
† p <0.05 vs. intrathymic saline and anti-CD3 mAb.

mAb, 60% of animals developed diabetes, and their glucose levels were similar to animals receiving Krebs-Hepes buffer and anti-CD3 mAb.

**Tolerance to MDSDM Can Be Abrogated by Splenocytes from Normal Mice.** To verify that tolerance had resulted from deletion or inactivation of islet antigen-reactive T cells from the repertoire of the mice, we performed two additional experiments. First, mice that had received intrathymic, STZ-treated islets and anti-CD3 mAb received an adoptive transfer of 40 × 10⁶ splenocytes from normal C57BL/KsJ mice on the day of the first injection of STZ (Table 4). All of these mice developed diabetes when given the five doses of STZ, and the glucose levels were similar to those of mice treated with intrathymic Krebs-Hepes buffer and anti-CD3 mAb. Second, after thymectomy (Fig. 1), individual mice protected from diabetes were given syngeneic spleen cells (n = 4) or saline (n = 3), and one or two injections of STZ (40 mg/kg). Mice that received splenocytes and STZ became hyperglycemic, whereas mice that received saline and STZ did not. Thus, susceptibility to MDSDM could be adoptively transferred to tolerant mice with splenocytes from normal animals.

**Discussion**

We have shown that intrathymic transfer of islets of Langhans and T cell regeneration can induce T cell tolerance to the development of autoimmune diabetes. Tolerance was specific for islets and was not due to general immunosuppression, since animals receiving the same anti-T cell mAb but without intrathymic islets developed diabetes similar to untreated mice, and animals with intrathymic islets rejected allogeneic skin grafts at a normal rate. Furthermore, the failure to develop hyperglycemia was not due to insulin production by the intrathymic islets that may have been protected from the autoimmune attack that occurs in the periphery, since thymectomy of the tolerized mice did not result in hyperglycemia. The number of islets placed into the thymuses was relatively small (about 200/mouse), and in other experiments we have been unable to reverse hyperglycemia in mice with toxic diabetes using this number of syngeneic normal islets. Rather, our results indicate that tolerance to autoimmune diabetes was the result of clonal deletion or inactivation of islet-reactive T cells.

The development of diabetes in the MDSDM model has previously been shown to be dependent on T cell responses (11-14, 21). Pretreatment of mice with anti-CD4, anti-CD8 (12), or treatment with anti-CD3 mAbs (21) can prevent induction of disease. In addition, hyperglycemia can be adaptively transferred with splenocytes from a diabetic animal to a recipient treated with a single dose of STZ (13). The events that occur after STZ treatment leading to the induction of diabetes are not clearly understood, and may involve expression of novel antigens on the surfaces of B cells or presentation of constitutive proteins to T cells. Although in our experiments STZ treatment of the islets in vitro was necessary to completely prevent diabetes, there was a small effect of non-STZ treated islets on the natural history of disease as well. These findings raise the possibility that islet-specific T cell antigen may be present in low levels or sequestered on normal B cells. With injury and possibly shedding of antigen, as might occur to some degree during isolation of the islets, or to a greater extent after STZ treatment, these antigens may become more available to the immune system. This concept is consistent with the observation that expression of foreign antigens on the surfaces of B cells does not result in an inflammatory response against islets (8, 9) unless accessory signals are provided. These signals can be provided, for example, by viral infection or possibly cytokines (22).
In the thymus, antigens shed from islets may be presented to developing T cells by APCs that are either bone marrow or islet derived. Although bone marrow-derived cells are thought to be primarily responsible for mediating clonal deletion in the thymus (1, 3, 4), extrathymic APCs have also been shown to be capable of mediating clonal deletion of immature CD4+CD8+ thymocytes in suspension (23). Thus, the cells that present islet antigen to the developing T cells may be found within the islets themselves, or may be host derived and can process antigens that are shed by STZ-treated islets. If, in fact, shed antigen is presented by thymic cells, this approach for induction of tolerance may not require histocompatibility of the host and antigen-bearing cell, since transplantation of MHC incompatible tissues can be performed easily into the thymus with a single treatment with anti-T cell antibody (15).

Treatment with anti-CD3 mAb was required to induce tolerance, and tolerance did not develop when islets were placed outside of the thymus. Our findings suggest that induction of nonresponsiveness to autoimmune diabetes was due to T cell maturation in the presence of islet antigens which was stimulated by anti-CD3 mAb treatment. A single injection of anti-CD3 mAb has been shown previously to completely remove mature T cells from the peripheral blood (16). The residual (~30%) T cells in the peripheral organs show profound functional impairments even after TCR reexpression has occurred. The repopulation of the peripheral organs with T cells most likely reflects accelerated maturation of T cell precursors through the thymus and emigration into the peripheral sites. T cells on which the TCR has been modulated as a result of anti-CD3 mAb treatment may reexpress this complex, but evidence from thymectomized animals (16) indicates that T cell repopulation occurs in half the time if a thymus is present. It has previously been shown by Posselt et al. (25) that a reduction in the precursor frequency of alloreactive T cells occurs when T cell development takes place in the presence of allogeneic islets in the thymus (15). While our work was in progress, Koevary and Blomberg (24) and Posselt et al. (25) have reported that autoimmune diabetes may be prevented by intrathymic transplantation of islets into young or neonatal BB/W rats. Thus, our studies and those previously reported support the notion that islet antigen-reactive T cells have been either depleted from the repertoire or inactivated as a consequence of interaction with foreign antigens in the thymus during T cell development. Mature T cells that have developed in a thymus that lacks islet cells abrogate this protective effect. Thus, potentially autoreactive, islet-specific T cells escape thymic deletion in normal C57BL/KsJ mice. Failure to delete these T cells during normal development may reflect the inaccessibility of islet antigens for antigen presentation possibility because the antigens are not shed or are in low concentration.

Clonal deletion or clonal anergy may be the outcome of interaction of T cells with antigen present in the thymic medulla. For example, Hammerling et al. (25) have recently found that T cells reactive with the alloantigen Kb may be rendered nonresponsive after encounter with the alloantigen in the thymic medulla, but do not necessarily undergo apoptosis. Because there is no specific phenotypic marker (i.e., TCR-V region gene) known as yet for the autoreactive T cells in this model, we cannot determine whether islet-reactive T cells have actually been deleted, or whether they are still present but are incapable of causing diabetes. Either clonal anergy or deletion of antigen-reactive T cells is consistent with our observation that responsiveness to MDSDM may be restored with normal spleen cells. However, our findings indicate that induction of regulatory ("suppressor") cells in the periphery is not responsible for the effect we have observed.

When removed from mice 5 wk after surgery, the thymi of mice receiving intrathymic islets still contained insulin-producing cells. The small number of islet cells persisting in the thymus does not likely represent a significant source of insulin, but may be important in maintaining the tolerant state. We do not know how long the tolerant state will persist after thymectomy, but it is likely that continual maintenance of tolerance will require that antigen remain in the thymus to ensure removal of antigen-reactive cells will occur as new T cells develop.

The ability to induce antigen-specific tolerance, especially tolerance towards disease-related antigens, represents an optimal approach for prevention of autoimmune diseases. We have shown that this may be induced in animals with a mature T cell repertoire. With this technique, only T cells specific for disease-relevant antigens are eliminated as a consequence of the manipulation, and other cellular immune functions are left intact. Thus, the ability to eliminate disease-relevant T cells in the absence of global immunosuppression represents a novel approach to disease-specific immunotherapy.

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