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Endogenous Oocyte Antigens Are Required for Rapid Induction and Progression of Autoimmune Ovarian Disease Following Day-3 Thymectomy

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Female (C57BL/6×A/J)F₁ mice undergoing thymectomy on day 3 after birth (d3tx) developed autoimmune ovarian disease (AOD) and autoimmune disease of the lacrimal gland. As both were prevented by normal adult CD25⁺ T cells, regulatory T cell depletion is responsible for d3tx diseases. AOD began as oophoritis at 3 wk. By 4 wk, AOD progressed to ovarian atrophy with autoantibody response against multiple oocyte Ag of early ontogeny. The requirement for immunogenic endogenous ovarian Ag was investigated in d3tx female mice, d3tx male mice, and d3tx neonatally ovariectomized (OX) females. At 8 wk, all mice had comparable response against multiple oocyte Ag of early ontogeny. The requirement for immunogenic endogenous ovarian Ag was investigated.

The prevention of autoimmune disease in normal individuals and the events leading to autoimmune disease occurrence are likely dependent on multiple and complex mechanisms. Recent studies on the spontaneous autoimmune diseases that follow perturbation of the normal immune system, such as in mice undergoing thymectomy on day 3 after birth (d3tx), have begun to reveal the physiological role of T cell subsets that guard against the pathogenic expression of autoreactive T cells in the normal rodent (1–3). d3tx in different inbred mice results in a high frequency of independent autoimmune diseases that target the ovaries, stomach, thyroid, lacrimal gland, prostate, and testis, and in the production of the respective organ-specific autoantibodies and pathogenic T cell responses (4–9). Importantly, the T cell dependent autoimmune diseases occur in inbred mice that are not genetically prone to development of spontaneous autoimmunity. Therefore, the regulatory mechanism being disrupted by d3tx is likely to be of physiological relevance and is generalizable to different self Ag present in many organs (10).

An imbalance of effector and regulatory T cells has been proposed as the basis of the d3tx autoimmune diseases for several reasons. A regulatory T cell population has been found in normal adult mice that expresses the IL-2 receptor α-chain (CD25) (11–13). The infusion of CD4⁺ CD25⁺ spleen T cells or CD4⁺CD8⁻CD25⁺ thymocytes from normal syngeneic adults completely blocks d3tx diseases (11, 14). In addition, the thymus derived CD25⁺ CD4 T cells are detected in the periphery after day 3 of life, thus this T cell population would be depleted or reduced by d3tx (12). Finally, CD25⁻ T cells that emigrate from the thymus before day 3 have pathogenic potentials in the absence of CD25⁺ T cells (12). For example, CD4⁺ T cells from 3-day-old, but not adult, euthymic BALB/c donors were found to transfer autoimmune diseases into syngeneic nu/nu recipients (12, 15).

In addition to elucidating the regulatory T cells and their mode of action, it is important to define the parameters surrounding the development of autoimmune diseases that follow regulatory T cell depletion. One parameter is the source of antigenic stimulus responsible for the spontaneous autoimmune response. Because d3tx disease induction does not follow deliberate Ag injection, a critical question is whether the response is initiated by exogenous Ag through molecular mimicry, or alternatively, represents the response of a dysregulated immune system driven by endogenous Ag? One might also ask whether endogenous Ag are required for the maintenance of the ongoing autoimmune response, and, would the response regress upon removal of the endogenous Ag? These questions are pertinent to the understanding of neonatal immune responses, the capacity of Ag from normal organs to initiate pathogenic autoimmune responses, self tolerance mechanism, and eventually the regulatory T cell action.

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For this study, we have taken advantage of autoimmune ovarian disease (AOD) that occurs at high frequency in the d3tx (C57BL/6×AJ)F1 (B6AF1) mice. Ovarian Ag is gender specific and its expression can be manipulated experimentally. As an example, ovarian Ag can be depleted surgically at any time of life. In mice without endogenous ovaries, viable syngeneic ovarian grafts provide a potential source of ovarian Ag stimulus and can be the target for AOD. Based on this model, we investigated the requirement and the duration of endogenous ovarian Ag stimulation necessary for the initiation and progression of AOD in d3tx mice. As an integral part of this investigation, we defined the nature and ontogeny of ovarian Ag relevant to AOD in the d3tx mice.

Materials and Methods

Animals and surgery

A breeding colony B6AF1 mice was established by mating C57BL/6 female and A/J male mice purchased from National Cancer Institute (Frederick, MD). Mice were kept and handled in accordance of approved National Institutes of Health guidelines. Thymectomy was performed on 3-day-old B6AF1 pups using the suction technique (16). Completeness of thymectomy was verified histologically, and mice with incomplete thymectomy were excluded from analysis. Ovarioctomy (OX) was performed by removing both ovaries through a posterior incision, under dissection microscopy. To implant ovaries, the ovary from a 6- to 8-wk-old B6AF1 mouse was inserted under the kidney capsule through a posterior incision. Neonatal mice were anesthetized by hypothermia or metofane and adult mice with trichromethanol.

Histological evaluation of ovarian and lacrimal gland inflammation

The ovaries and lacrimal glands were fixed in Bouin’s solution and embedded in paraffin. 5-μm sections were stained with hematoxylin and eosin. The extent of ovarian pathology was determined as unknown samples. Ovarian inflammation and ovarian atrophy were graded separately each from 1 to 4, as described previously (16). Grade 1 inflammation consisted of 1–2 foci of inflammatory cells, consisting of monocytes, lymphocytes, and a few granulocytes, usually at the ovarian hilum. Grade 4 represented diffuse inflammation throughout the ovary involving both follicles and interstitial space. Grades 2 and 3 represented intermediate inflammation of incremental severity. Atrophy was evidenced as loss of growing or mature ovarian follicles, hypertrophy, leuteinization of interstitial cells, and eventually, loss of primordial oocytes. Lacrimal gland inflammation was graded from 1 to 4. Grade 1 lacrimalitis contained <1-2 foci of periductal mononuclear inflammatory cells. Grade 4 disease exhibited atrophy of lacrimal acini and severe lymphoid infiltration with destruction of lacrimal ducts. Grades 2 and 3 lacrimalitis represented incremental intermediate degrees of inflammation between grades 1 and 4.

Detection of ovarian Ags and Abs by immunofluorescence and immunoblot

Frozen sections of ovaries from naive B6AF1 or BALB/c scid mice were fixed in acetone, and processed for indirect immunofluorescence. All mouse sera were tested at a 1:50 dilution, and FITC labeled goat Ab to mouse IgG was used as the second Ab. The slides were viewed and photographed with an Olympus fluorescence microscope (New Hyde Park, NY). Absence of endogenous IgG in young BALB/c mice was determined at the University of Virginia facility (Charlottesville, VA).

Spleen cells were dissociated and filtered through nylon mesh, and erythrocytes were lysed in hypotonic 0.1% ammonium chloride. After washing in RPMI 1640, the cells were suspended in PBS. Each recipient (7–7.5 days old) was injected i.p. with 35 × 10^6 viable cells in a 100-μl volume, and their ovaries were studied histologically 10 days later.

Isolation and transfer of CD25^+ CD4^+ T cells to prevent AOD in d3tx mice

Lymphoid cells were obtained from axillary, inguinal, brachial, and cervical lymph nodes, and from the spleen of 8-wk-old normal B6AF1 female mice. They were enriched for CD4^+ cells using the CD4 enrichment column (R&D Systems, Minneapolis, MN). CD4^+ T cells were then labeled with biotinylated anti-CD25 Ab (PharMingen, San Diego, CA), incubated with streptavidin–iron beads (Miltenyi Biotec, Auburn, CA), and isolated on a magnetic column. Seventy percent of the cell preparation were CD25^+ T cells (data not shown). A total of 2 × 10^6 cells in 100 μL PBS were injected i.p. into 7-day-old d3tx mice.

Results

Progression of AOD in d3tx female B6AF1 mice

We first established the prevalence and the time of onset of the different components of AOD in female B6AF1 mice thymectomized at the University of Virginia facility (Charlottesville, VA). Ovarian inflammation (ophoritis), detected in occasional mice at 2 wk, reached ~70% by 3 wk (Table I). At this time, ~10% of the ovaries were atrophic. In parallel, the spleen cells from 3-wk-old but not 2-wk-old d3tx donors transferred oophoritis to young recipients. The severity of AOD then escalated in the ensuing week. Although oocyte autoantibodies were not detected at 3 wk, most d3tx mice had oocyte autoantibodies in their sera by 4 wk. At the same time, the ovaries of 40% of the animals became atrophic, some with residual oophoritis. This sequence of events occurred in d3tx mice from different B6AF1 colonies although the exact time of onset could differ by 1–2 wk (8, 19, 20).

Endogenous ovarian Ag is required for AOD occurrence in d3tx mice

To determine the requirement of endogenous ovaries as a source of antigenic stimulus for ovarian autoimmune response, the responses
of female and male mice were compared (Table II). They were thymectomized on day 3, engrafted with a 6-wk-old B6AF1 ovary at 6 wk, and studied at 8 wk. Female mice developed AOD in the ovarian graft and serum autoantibodies to oocytes (Fig. 1A). In contrast, the ovarian grafts of male mice that lack endogenous ovaries were normal and their sera were free of detectable oocyte autoantibodies when they were studied at 2 wk (Fig. 1B), or at 6 wk after ovarian engraftment (one of nine had focal AOD). The requirement of endogenous ovaries for AOD induction was confirmed by the absence of AOD in d3tx females that were OX at day 3 of age (Table II). In contrast to AOD, autoimmune lacrimalitis occurred in d3tx mice regardless of whether they had endogenous ovaries or not (Table II). Thus, loss of the endogenous ovaries does not impact on pathogenic autoimmune responses in general.

As evidence that regulatory T cell depletion was the basis for the autoimmune diseases, both AOD and autoimmune lacrimalitis were found to be inhibited by the transfer of CD4+ CD25+ T cells from the normal B6AF1 adult females (Table II). In addition, autoimmunity was absent in sham thymectomized mice. The findings indicate that endogenous ovarian Ag is required for AOD development in the d3tx female mice. In the next study, we analyze the nature of the ovarian autoantigens and the ontogeny of their expression.

The nature and ontogeny of endogenous ovarian Ag

The majority of serum autoantibodies in the d3tx B6AF1 mice reacted with the intracellular Ags of growing and mature oocytes detected by immunofluorescence on ovarian sections (Fig. 2). By immunoblot, the autoantibodies were found to react with three distinct oocyte Ag with the apparent molecular mass of 110, 90, and 75 kDa (Fig. 2B). Among 38 sera from B6AF1 mice with oocyte Ab detected by immunofluorescence, 26 (69%) were also detected by immunoblot, and among them, 50% reacted with the 110-kDa oocyte Ag, 37% reacted with the 75-kDa oocyte Ag, and 18% reacted with the 90-kDa oocyte Ag.

The sera of 10 d3tx mice were also evaluated weekly for oocyte Ab from 6–10 wk. In four mice, autoantibodies to oocyte was detected only by immunofluorescence during the 4-wk period. In the remaining six d3tx mice, the initial Abs were detected by immunofluorescence of which three also recognized the 110-kDa oocyte Ag by immunoblot. This was followed in the next 1–3 wk by the emergence of Ab to the 110-kDa oocyte Ag in two additional mice, and new Ab to the 90-kDa oocyte Ag and the 75-kDa oocyte Ag in three and two mice, respectively (data not shown). Thus the earliest autoantibodies in the sera of d3tx B6AF1 were directed to native determinants of the oocyte cytoplasmic Ag including those of the 110-kDa component.

We next determined the ontogeny of oocyte Ag to establish the earliest age when endogenous antigenic stimulus could occur. As shown in Fig. 2, D, and E, oocyte Ag was detected in normal BALB/c scid mice at 1 day (6 of 8 sera) or 5 days (11 of 12 sera) of age by immunofluorescence. This finding parallels the detection of mRNA for the major 110-kDa Ag by RT-PCR on the day of birth (Fig. 2C). Thus ovarian autoAg that react with Abs, produced by d3tx mice, are detectable close to the day of birth in female pups.

To determine whether ovarian autoAg is recognized by pathogenic T cells from the d3tx mice, spleen cells from 6-wk-old d3tx mice were injected i.p. into neonatal recipients on the day of birth. Ovarian inflammation was detected 3 days later, whereas spleen cells from sham thymectomized mice did not transfer disease (Fig. 2, F and G). Inflammation was not detected in organs outside the ovaries. Thus, within the first 3 days of life, ovarian oocyte Ag was processed and presented by ovarian APCs that can be recognized by pathogenic oocyte-specific T cells. The expression of the oocyte Ag early in life indicates that immunogenic ovarian Ag are available to trigger an autoimmune response in neonatal mice.

Endogenous ovarian antigenic stimulus for 3 wk is required for induction of oophoritis

We next determined the duration of endogenous antigenic stimulation required for the induction of AOD and autoantibody response. By removing the ovaries from the d3tx female mice at 2,
3, or 4 wk, and assuming that Ag stimulation occurs from the day of thymectomy (day 3), we allowed endogenous ovarian antigenic stimulation to persist for 1.5, 2.5, or 3.5 wk. Each mouse was then engrafted with an adult ovary 2 wk after OX, and the ovarian graft was studied 2 wk later at 6, 7, or 8 wk of age, respectively.

Of the d3tx mice OX at 2 wk, none developed AOD or autoantibody response although most animals in this group developed autoimmune lacrimalitis (Table III). Therefore, consistent with the time of onset of AOD in the d3tx mice, ovarian Ag stimulation for 1.5 wk is not sufficient to elicit AOD in the d3tx mice.

In contrast to mice OX at 2 wk, 85% of the mice OX at 3 wk developed severe oophoritis and ovarian atrophy by 5 wk (Table III). Because these responses were indistinguishable from the responses of mice that were OX at 4 wk (and studied at 6 wk; Table III), we concluded that Ag stimulation from endogenous ovaries for 2.5 wk from d3tx was sufficient to elicit maximum autoimmune response and AOD.

Regardless of the age when the OX was performed, all d3tx mice developed high incidences of lacrimalitis (Table III). Thus ovarian ablation impacted specifically on the ovarian immune response.

**Ovarian antigenic stimulus is required for progression from oophoritis to ovarian atrophy and autoantibody response**

Although d3tx mice OX at 3 wk and engrafted at 5 wk developed severe AOD that progressed to atrophy and oocyte Ab production (Table III), it was surprising that the ovarian disease of d3tx mice studied at 3 wk had oophoritis without atrophy or autoantibodies response (Table II). In addition, mice that were OX at 3 wk and engrafted at 12 wk had significantly reduced incidences and severity of AOD, including oophoritis \((p < 0.05)\) and oocyte autoantibodies \((p < 0.01;\) Table IV). Both observations suggest that endogenous ovarian Ag derived from ovarian grafts are required for the maintenance and progression of the autoimmune response associated with severe AOD. In the following experiments, we test the hypothesis that endogenous ovarian Ag is also required for disease progression.

Groups of d3tx mice were OX at 3, 4, or 5 wk. In each group, we compared the immunopathology of ovaries that were engrafted at 2 wk vs 3 wk after the mice were OX (Table IV). Indeed, the ovarian inflammation was much more severe in ovaries engrafted at 5 wk (Fig. 1C) than those engrafted at 6 wk (Fig. 1D) \((p = 0.02)\). Moreover, the progression from oophoritis to atrophy and induction of autoantibodies response were largely halted in mice that were devoid of ovaries for 3 wk rather than 2 wk \((p < 0.01)\).

A similar finding was noted in d3tx mice OX at 4 wk, with a higher incidence of atrophy in the ovaries engrafted at 6 wk rather than 7 wk \((p < 0.05)\). The difference was less prominent in mice OX at 5 wk. Although there was a significant reduction in autoantibodies response in mice engrafted at 8 wk instead of 7 wk \((p < 0.001)\), the incidence of oophoritis and atrophy in ovaries engrafted at 7 or 8 wk were comparable. These data, together, indicate that ovarian Ag from endogenous ovaries or ovarian graft is required to stimulate full progression of AOD induced by d3tx.

**Discussion**

This study has investigated the requirement of endogenous Ag in the initiation and propagation of autoimmune response and AOD.
in d3tx mice. Male mice and OX female mice both lack endogenous ovarian Ag and neither group developed oophoritis or produced detectable autoantibodies to oocyte Ag following d3tx. Thus endogenous ovarian Ag is required for disease induction. The difference in AOD between d3tx male and female mice could be due to gender difference or other OX effects. However, this is unlikely because all d3tx animals developed a high prevalence of autoimmune lacrimalitis, a disease that is not gender specific. The present finding is supported by the earlier studies wherein spleen cells from d3tx mice without endogenous ovaries failed to transfer AOD to young recipients (6, 20). The requirement of endogenous Ag has also been demonstrated for spontaneous autoimmune thyroiditis in obese strain chicken (21), and for autoimmune diabetes in the nonobese diabetic (NOD) mice (22). In the latter, alloxan treatment destroyed the pancreatic islet β cells of NOD mice, and their T cells did not transfer diabetes to irradiated male NOD recipients. A more recent study with a transgenic diabeticogenic peptide-specific TCR indicated that T cell activation in the regional lymph node was the first observable pathogenic event in NOD mice (23). However, these studies did not investigate the duration of Ag stimulation required for disease induction, nor did they address the impact of endogenous Ag on autoimmune disease progression.

The present study demonstrated clearly that Ag provided by an ovarian graft is required for progression of AOD. As shown in Tables III and IV, although Ag exposure for the first 2.5 wk in the d3tx mice is sufficient for AOD development, including oocyte Ab and atrophy, this maximum response was observed only when the ovaries were engrafted 2 wk after removal of the endogenous ovaries. When ovarian engraftment was delayed by an extra week (i.e., ovarian engraftment at 3 instead of 2 wk after OX), oophoritis was no longer accompanied by ovarian atrophy or autoantibodies response. Thus disease progression to atrophy and autoantibody response was halted when persistent Ag stimulation was interrupted by 3 instead of 2 wk. This trend was also observed in mice OX at 4 wk; and in this case, more severe disease was observed in mice engrafted at 6 rather than 7 wk. Indeed, the disease may regress upon further withdrawal of Ag stimulus. For example, in d3tx mice that were OX at 3 wk and studied at 12 wk, the incidence of oophoritis was significantly reduced (p < 0.05; Table IV). In mice OX at 5 wk, reduction in subsequent Ag exposure from the ovarian graft also reduced autoantibodies response but had little effect on progression from oophoritis to atrophy. It is known that the number of oocyte progenitors is finite, and when they are lost upon ovarian atrophy, recovery from AOD might not be possible. We have recently obtained direct evidence for the capacity of an ovarian graft to confer antigenic stimulation. Male mice injected with a T cell epitope of the ovarian zona pellucida Ag ZP3 (24) did not produce Ab to distant B cell epitopes of ZP3 unless they were engrafted with an ovary (P. Pramoonjago, C. Sharp, and K. S. K. Tung, unpublished observation). Therefore, we conclude that endogenous or engrafted ovarian Ag is required for AOD induction as well as for AOD progression.

The presence of the ovarian Ag for 3 wk from birth was required for maximum disease induction, whereas Ag exposure for 2 wk did not induce autoimmune disease (Table III). Assuming that endogenous Ag stimulation occurs on day 3 when the mice were thymectomized, the finding suggests that Ag stimulation for the first 2.5 wk after thymectomy is sufficient to initiate a maximum T cell response. This is consistent with AOD transfer by spleen cells from d3tx mice at 3 wk but not at 2 wk of age (Table I). This
Duration is comparable to the time required for induction of autoimmune diseases by immunization with tissue peptides in complete adjuvant. The rapid induction of autoimmune response by endogenous Ag in the neonatal mice without the use of adjuvant is quite surprising and has several interesting and important implications. It suggests that endogenous ovarian Ag are available and are presentable in immunogenic form by APC capable of activating neonatal T cells that are presumably naive. It also suggests that neonatal T cells in the d3tx mice have full capacity to mount a pathogenic autoimmune response. Thus tight regulation must normally guard against spontaneous autoimmune disease in the neonatal mice, and this begs the question as to how the regulation is abrogated by CD25+ regulatory T cell depletion.

The lack of autoimmune response in the d3tx mice studied at 2 wk, or in the d3tx mice OX at 2 wk and engrafted at 4 wk, is not due to a late ontogeny of oocyte Ag. In this study, we have documented the multiple oocyte Ag to which the autoimmune responses of d3tx B6AF1 mice are directed. The 110-kDa oocyte Ag, which may elicit the earliest autoimmune response, has been characterized by Tong and Nelson (17) as a prefertilization oocyte protein required for blastocyst formation after fertilization (25). In this study, we have shown clearly that the transcript for this oocyte autoantigen is detectable in B6AF1 ovaries on the day of birth, and that oocyte Ag are recognized by autoantibodies 1 day later, and by CD4 T cells as early as 3 days later. In the neonatal week, a large fraction of oocytes are normally eliminated by apoptosis from normal ovaries. For example, an C57BL/6 ovary contains 60,000 oocytes on day 0, and the number is reduced to 20,000 by day 3 (26) and, therefore, are available to invoke the autoimmune responses observed in the d3tx mice.

With regard to the availability of autoreactive effector T cells, it has been shown that neonatal T cells are immunocompetent (28, 29). Moreover, when transferred to nude recipients, the spleen cells from normal 3-day-old BALB/c mice induce ovarian and gastric autoimmune diseases spontaneously (12, 15). Therefore, the accessibility of endogenous Ag and the competence of pathogenic T cells are not limiting for induction of spontaneous autoimmune disease in the neonatal mice. A more likely limiting factor is the immaturity of the neonatal APC.

Macrophages from neonatal mice have minimal MHC II expression (30). A recent study indicated that B cells and plastic adherent cells from normal neonatal spleens presented Ag to memory T cells at ~30% of the adult level in the first week (31). However, it has also been shown that the immature neonatal APC function might be modified, for example, after adjuvant or CD40 agonist Ab treatment. Thus autoimmune responses and autoimmune diseases were elicited in the neonatal mice by injection of self peptides in complete or incomplete adjuvant (32–34); and CD40 Ab was found to abrogate induction of neonatal tolerance to alloantigen (35). In the d3tx mice, because the self Ag-driven autoimmune disease is inhibitable by CD25+ T cells, it is possible that deletion of the regulatory T cell population by d3tx somehow capacitates neonatal APC to present ovarian self Ag to naive autoreactive T cells. Recent literature supports this contention. For example, CD4+ CD25+ T cells (36) and anergic regulatory T cells (37) have been reported to down-regulate expression of costimulatory molecules on APC and to reduce APC function. In addition, cytokines produced by T cells, including IL-10 (38, 39) and TGFβ (40), also

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### Table III. Duration of endogenous antigenic stimulus required for induction of AOD and pathogenic T cell and autoantibody responses in the d3tx mice

<table>
<thead>
<tr>
<th>Age (wk)</th>
<th>Incidence of oophoritis (%)</th>
<th>Oophoritis severity (1–4) (mean ± SE)</th>
<th>Atrophy in mice with oophoritis (%)</th>
<th>Serum oocyte Ab (%)</th>
<th>Incidence of Atrophy (%)</th>
<th>Mean severity (1–4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bilateral OX</td>
<td>Ovarian implantation</td>
<td>2</td>
<td>4</td>
<td>0/7</td>
<td>0/7</td>
<td>0/7</td>
</tr>
<tr>
<td>3</td>
<td>5</td>
<td>7/8 (88)</td>
<td>3.7 ± 0.29</td>
<td>6/7 (86)</td>
<td>0/7</td>
<td>0/7</td>
</tr>
<tr>
<td>4</td>
<td>6</td>
<td>9/10 (90)</td>
<td>3.5 ± 0.29</td>
<td>7/9 (77)</td>
<td>9/12 (75)</td>
<td>9/11 (45)</td>
</tr>
</tbody>
</table>

* All B6AF1 female mice were subjected to d3tx and later OX. Two weeks after OX, each mouse received an ovarian graft from 6-wk-old B6AF1 female mice.

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### Table IV. Ovarian antigenic stimulus is required for the progression from oophoritis to ovarian atrophy and oocyte autoantibody response in the d3tx mice

<table>
<thead>
<tr>
<th>Age of Mice (wk)</th>
<th>Incidence of oophoritis (%)</th>
<th>Oophoritis severity index (1–4) (mean ± SE)</th>
<th>Atrophy in mice with oophoritis (%)</th>
<th>Serum oocyte Ab (%)</th>
<th>Incidence of Atrophy (%)</th>
<th>Mean severity (1–4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bilateral OX</td>
<td>Ovarian implantation</td>
<td>3</td>
<td>5</td>
<td>7/8 (88)</td>
<td>3.9 ± 0.14</td>
<td>6/7 (86)</td>
</tr>
<tr>
<td>4</td>
<td>6</td>
<td>8/11 (73)</td>
<td>3.0 ± 0.27</td>
<td>2/8 (25)</td>
<td>4/11 (36)</td>
<td>9/11 (82)</td>
</tr>
</tbody>
</table>

* See footnote in Table III. See text for statistical differences in data between experimental groups.
inhibit APC function. Powrie and colleagues (41, 42) have provided in vivo evidence that these two cytokines are involved in the control of T cell-mediated colitis by regulatory T cells that express CD45RB bright and CD25. Therefore, CD25+ T cells may normally regulate APC function directly, or via the secretion of IL-10 and/or TGFβ.

In summary, although there is considerable evidence that autoimmune disease can be elicited by exogenous Ag through molecular mimicity at the level of T or B cell responses (43, 44), research based on the AOD and other spontaneous autoimmune models emphasizes a more critical role for endogenous Ag in the prevention, induction, and maintenance of pathogenic autoimmune responses. In a recent study, we documented the requirement of endogenous Ag for the maintenance of physiological tolerance to ovarian self Ag (45). Now, in the d3tx context, we have demonstrated that ovarian endogenous Ag can also trigger and sustain AOD progression. To accommodate this apparent paradox, we propose that endogenous Ag stimulation occurs normally, and that the outcome is dependent on the integrity and the continuous operation of the immune regulatory mechanisms.

Acknowledgments

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References


