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Retargeting T Cell-Mediated Inflammation: A New Perspective on Autoantibody Action[1]

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To understand the pathogenesis of organ-specific autoimmune disease requires an appreciation of how the T cell-mediated inflammation is targeted, and how the organ function is compromised. In this study, autoantibody was documented to influence both of these parameters by modulating the distribution of T cell-mediated inflammation. The murine autoimmune ovarian disease is induced by immunization with the ZP3$^{330-342}$ peptide of the ovarian zona pellucida 3 glycoprotein, ZP3. Passively transferred or actively induced Ab to ZP3$^{335-342}$ bound to the zona pellucida in the functional and degenerative ovarian follicles, and the ovaries remained histologically normal. Transfer of ZP3$^{330-342}$ peptide-specific T cells targeted the degenerative follicles and spared the functional follicles, and the resultant interstitial oophoritis was associated with unimpaired ovarian function. Unexpectedly, the coexistence of ZP3$^{330-342}$ peptide-specific T cells and zona-bound autoantibody led to a dramatic translocation of the ovarian inflammation to the growing and mature ovarian follicles, with destruction of the ovarian functional unit. Ab retargeted both Th1-induced mononuclear inflammation and Th2-induced eosinophilic inflammation, and retargeting was induced by murine and rat polyclonal Abs to multiple distinct native B cell determinants of the zona pellucida. Therefore, by reacting with the native determinants in tissue Ag, Ab alters the distribution of T cell-mediated inflammation, and results in destruction of the functional units of the target organ. We propose that this is a clinically important and previously unappreciated element of Ab action in autoimmune disease. ***The Journal of Immunology,*** **2000, 164: 5251–5257.**

Autoreactive T cells are pivotal in the pathogenesis of organ-specific autoimmune disease. Although proinflammatory T cells can induce tissue inflammation, inflammation alone may not result in functional loss of the target organ. This is illustrated by perivascular cuffing of mononuclear cells in nonparalytic experimental allergic encephalomyelitis (EAE)[4] (1) and periinsulitis in nondiabetic nonobese mice (2). In autoimmune ovarian disease (AOD) mediated by immune response to the murine ZP3$^{330-342}$ self peptide (pZP3), intense ovarian interstitial inflammation transferred by CD4$^+$ T cells is compatible with normal ovarian function and normal fertility (3). If loss of organ function requires direct T cell-mediated injury of the functional unit of the diseased organ, then one might expect the inflammatory cells and/or their cytokines to target and injure the myelin sheath, the pancreatic islet β cells, and the growing and mature ovarian follicles, respectively. Previous investigations have suggested that the distribution of autoimmune inflammation can be influenced by a number of factors, including the presence of appropriate T cell subsets (4), activated macrophages (5), and proinflammatory cytokines (6, 7). It may also be affected by the location of MHC class II expression (8, 9), tissue barrier that affects accessibility of T cells to the target Ag (9–11), and changes in the target organ (12, 13). In the present study, we provide evidence that autoantibody can target T cell injury to the ovarian follicles and eliminates the functional unit of the ovary.

This investigation was aided by several attributes of the AOD model. The self peptide that elicits AOD contains well-defined T cell epitope and native B cell epitope, with which selective T cell and Ab responses and their effects can be independently analyzed. The ovarian zona pellucida (ZP) is a well-defined antigenic structure accessible to circulating Ab, and therefore precise detection of Ab to the ZP in vivo is possible. Most important, the ovarian ZP3 is found in two distinct anatomical locations: one, as native ZP surrounding the oocytes of the functional growing and mature follicles, and the other, as ZP in degenerating oocytes within atretic follicles. The relative extent of inflammation affecting each of the anatomical sites therefore provides an index of targeting of inflammation and tissue injury within the ovary.

The result indicates that autoantibody binding to the ZP of the functional growing and mature ovarian follicles is associated with a redistribution of T cell-mediated inflammation from the nonfunctional atretic follicles to the functional unit of the ovary. Inflammation in the ovarian follicles then leads to their destruction. The finding has led to a new concept that autoantibody can influence the clinical outcome of a T cell-mediated autoimmune disease by focusing for destruction the functional unit of the diseased organ.

Materials and Methods

Immunization and antigenic peptide

(C57BL/6 × A/J)F_1 (B6AF_1) and BALB/cBy female mice, obtained from The Jackson Laboratory (Bar Harbor, ME), were studied at 6–8 wk of age. Peptide Ags, dissolved in milli-Q water at 1 mM and sterilized by ultrafiltration, were emulsified in an equal volume of CFA. Each mouse received 0.1 ml of the mixture that contains 50 nmol of peptide, in one

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[4] Abbreviations used in this paper: EAE, experimental autoimmune encephalomyelitis; AOD, autoimmune ovarian disease; CP, chimeric peptide; pZP3, ZP3$^{330-342}$ peptide; ZP, zona pellucida.

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footpad and a s.c. site, and was examined in 14 days or as indicated. Female Lewis rats from National Cancer Institute were immunized with 0.3 ml of peptide-CFA mixture at s.c. sites, and boosted with same peptide in IFA 1 mo later. Rats were bled by cardiac puncture 2 wk after boosting.

All peptides were synthesized by an automatic peptide synthesizer (Gilson, Middleton, WI), and purified by HPLC on a C18 reverse phase column (Waters, Millford, MA). All peptides exceeded 95% in purity. Amino acid composition was verified by amino acid analysis. The amino acid sequences of the peptides used in this study are as follows: $ZP3^{330-342}$, NS SSSQFQIHGPR; $ZP3^{335-342}$, QFQIHGPR; $ZP3^{330-340}$, NSSSSQFQIHG; chimeric peptide (CP)1, NCAYKTTQANKAAAAAQFQIHGPR; CP2, NCAYKTTQANKQAQIHGPR (14).

Ovarian histopathology and immunohistology

Ovaries were fixed in the Bouin's fixative and embedded in paraffin. Five-micron-thick serial sections were stained with hematoxylin and eosin. Ovarian inflammation was graded with increasing severity from 1 to 4, as described (14), and the extent of inflammation in the ovarian interstitium, the growing follicles, and the mature follicles was graded separately. Ovarian atrophy was graded as follows: grade 1, focal loss of growing and/or mature follicular oocytes as demonstrated by the appearance of granulosa cell clusters without oocytes; grade 3, complete loss of follicular oocytes and an increase in interstitial ovarian cells; grade 2, between grade 1 and grade 2. All histology results were interpreted as unknown samples. For immunohistology, ovaries were fixed in 4% paraformaldehyde, transferred to 30% sucrose in PBS, and embedded in the OCT compound. Frozen sections were stained by immunoperoxidase to detect T cells, macrophages, and MHC class II. Briefly, sections were blocked in sequence by 10% normal goat serum, biotin, and avidin (kit from Vector Laboratory, Burlingame, CA). A rat mAb to mouse CD4 (GK1.5) or hamster mAb to CD3 (145-2C11) was incubated with the sections for 1 h, followed by thorough rinsing with PBS. The sections were further incubated with biotin-labeled goat anti-rat IgG or goat anti-hamster IgG for 45 min (1:100; Vector Laboratory), followed by ABC complex for 30 min (Vector Laboratory). A diaminobenzidine-based kit (BioGenex, San Ramon, CA) was used to visualize the immunoreactant, and osmium tetroxide (0.05% in PBS) was used to intensify the staining. Finally, the sections were counterstained with methylene blue. A similar method was used to identify the macrophages using rat mAb F4/80 and MHC II (14).

Production and transfer of monospecific Abs to ZP3 and ZP2

Mouse polyclonal monospecific Abs to $ZP3^{334-342}$ were generated as previously described (15). Briefly, BALB/cBy mice were immunized with a mixture of CFA and CP1 peptide, which contains the T cell epitope of bovine RNase (94–104) and the native B cell epitope $ZP3^{335-342}$ (QFQIHGPR), and boosted with the same peptide in IFA 4 wk later. Mice were then inoculated by i.p. injection with 10^6 of SP2/0 myeloma cells 7 days later. Ascites were aspirated from the peritoneal cavity. Serum was also collected when mice were sacrificed. Abs were titered by indirect immunofluorescence on normal frozen ovarian sections, and by the ELISA using peptide $ZP3^{330-342}$ as Ag (16). Ab titers were expressed as the reciprocal dilutions of the endpoint fluorescence. IgG subclasses of the Abs in ascites were semiquantitated by immunofluorescence, and all four subclasses were detectable, with IgG1 and IgG2a being the most abundant. Ascites or sera with high Ab titer (>3200) were pooled, aliquoted, and stored at −80°C until use. Rat anti-ZP3 antisera were made in female Lewis rats by immunization with the CP1 in CFA. A rat IgG2a mAb (IE-10) that recognizes the B cell epitope $ZP3^{335-342}$ was prepared as ascites in BALB/c SCID mice (titer 1:200,000; 5 mg/ml). Ab to $ZP3^{335-342}$ in sera or ascites (0.3–0.5 ml) was infused, i.p., into each recipient. The presence of transferred Ab in the recipient's serum was confirmed by ELISA using peptide $ZP3^{330-342}$ as Ag, or by direct immunofluorescence detection of rat IgG on ovarian ZP of the Ab recipient (16). A similar chimeric peptide approach was used to elicit Ab to the murine $ZP3^{171-180}$ and to the murine $ZP2^{121-127}$ B cell epitope (17, 18).

Generate and transfer ovarian autoimmune disease with ZP3-specific T cell lines

The method for generating ZP3-specific T cell lines has been described previously (14). Briefly, lymphocytes were obtained from the draining lymph nodes of mice immunized with $ZP3^{330-342}$ in CFA 14 days earlier. The cells were suspended at 10^6 cells/ml in complete DMEM medium (Life Technologies, Grant Island, NY) and stimulated in the presence of 10 mM of $ZP3^{330-342}$ peptide and 3×10^6 cells/ml of irradiated (2000 rad) syngeneic spleen cells as APC. Four days later, T cell blasts were harvested on Ficoll gradient, and rested for 7–10 days. The stimulation and resting cycles were repeated until a stable cell line was obtained. The T cell lines used in this study were: 1) the 386 line that produced typical Th1 T cell cytokines, and 2) the ZP3-A line, which produced IL-4 and IL-5 with little IFN-γ. A total of 10×10^6 recently activated T cells was suspended in 0.5 ml protein-free PBS, and injected i.p. in each syngeneic recipient, and both cell lines uniformly transferred disease to naive recipients (14) (P. Alard et al., manuscript in preparation).

Treatment with CTLA4-Ig

Human CTLA4-Ig and L6 were a generous gift from Dr. J. A. Ledbetter (Bristol-Myers Squibb, Seattle, WA). CTLA4-Ig (200 μg/injection) was administrated by i.p. injection on days 0, 2, 4, and 10 after immunization with $ZP3^{330-342}$. L6, chimeric protein containing human Fcγ and a murine V region against a human carcinoma Ag, was used as an isotype-matched control.

Results

Distribution of oophoritis induced by pZP3-specific T cells is confined to the ovarian atretic follicles

Two types of ovarian follicles are identified within the normal mouse ovaries: 1) the growing/mature ovarian follicles, and 2) the atretic follicles (Fig. 1*A*). When pZP3-specific $CD4^+$ T cells were transferred to normal recipients, inflammation developed exclusively in atretic follicles located in the ovarian interstitium, and spared the growing and mature follicles (Fig. 1*B*). The inflamed atretic follicles contained clusters of CD4 T cells and MHC II^+, $F4/80^+$ macrophages (Fig. 1, *C* and *D*). Predominantly interstitial oophoritis was found in recipients of five independent pZP3-specific T cell lines, regardless of whether the activated T cells produced predominantly IL-2 and IFN-γ, or predominantly IL-5 and IL-13 (Fig. 2) (14) (P. Alard, et al., in preparation). The interstitial distribution of oophoritis in the T cell recipients persisted over several weeks, and it was independent of the dose of the transferred T cells (data not shown). Recent study indicated that severe interstitial oophoritis in the T cell recipients occurs without impairment of ovarian function (3).

Inflammation in ovarian follicles correlates with autoantibody to ZP in AOD induced by pZP3 immunization

In contrast to oophoritis in T cell recipients, the ovarian inflammation in mice injected with pZP3 in CFA was detected in the ovarian interstitium (interstitial oophoritis) and also in the growing and mature ovarian follicles (follicular oophoritis). The histopathology of follicular oophoritis is illustrated in Fig. 1, *E–H*. Unlike AOD that followed adoptive transfer of pZP3 T cell line (Fig. 1*D*, *inset*), ZP Ab was detected in the ovaries following pZP3 immunization (Fig. 1*H*, *inset*). This suggests that ZP Ab may play a role in the pathogenesis of follicular oophoritis. Indeed, all but 2 of 104 among 229 mice had both follicular oophoritis and autoantibody to ZP (Table I); moreover, the serum ZP autoantibody titer correlated positively with severity of follicular oophoritis in mice with oophoritis ($p < 10^{-4}$).

The association between ZP Ab and follicular oophoritis was also demonstrated in mice immunized with pZP3 treated with CTLA4-Ig to block the CD28/B7 costimulation pathway. We showed previously that mice treated with CTLA4-Ig and immunized with pZP3 in CFA no longer produced ZP Abs; however, T cell response to pZP3 was reduced, but remained detectable, and the mice developed oophoritis, although it was less severe (19). Further analysis of the CTLA4-Ig-treated mice now indicates that the ovarian inflammation was confined to the atretic follicles (7 of 11) and not in the growing or mature follicles (0 of 11) ($p < 10^{-5}$) (Table II). In contrast, control mice immunized with pZP3 and receiving L6-produced ZP Abs developed both follicular oophoritis (6 of 13) and interstitial oophoritis (9 of 13) ($p = 0.71$).

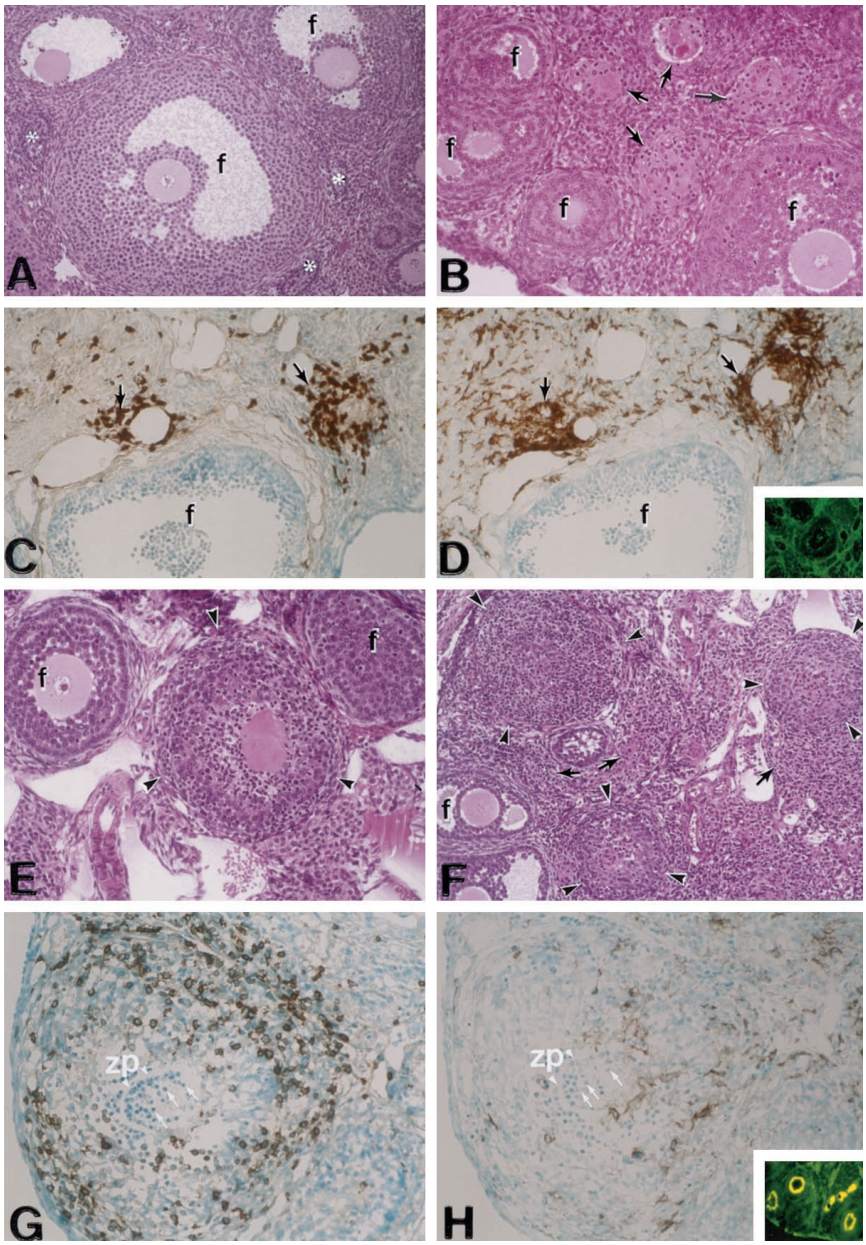

FIGURE 1. Histology and immunohistology of a normal ovary and ovaries with autoimmune oophoritis. *A*, Histology of a normal $B6AF_1$ mouse ovary that contains growing and mature follicles (f) and atretic follicles (asterisks). *B*, Ovarian histology of a mouse injected with pZP3-specific T cells, with numerous monocytic graulomas (arrows) located in atretic follicles of the interstitium, sparing the growing and mature follicles (f). *C* and *D*, Immunoperoxidase staining that colocalizes T cells (CD3) (*C*) and MHC II^+ cells (*D*) in two atretic follicles (arrows) on adjacent sections of an ovary from a pZP3-specific T cell recipient. The normal follicle (f) is unaffected. *Inset* in *D* shows negative immunofluorescence staining for mouse IgG in the ovarian ZP of the T cell recipient. *E*, Ovarian histology of a mouse injected with CP2 in CFA and later received pZP3-specific T cells, with inflammation that affects a growing follicle (arrowhead) and spares two other growing follicles (f). *F*, Ovarian histology of a mouse that received both mouse polyclonal Ab to ZP3 and a pZP3-specific T cell line. Inflammation affected three growing follicles (arrowheads), but spared two other follicles (f). *G* and *H*, Immunoperoxidase staining that colocalizes T cells (CD3) (*G*) and MHC II^+ cells (*H*) in mature ovarian follicles from a mouse that received both murine Ab to pZP3 and pZP3-specific T cells. Leukocytes (arrows) have replaced the oocytes inside the ZP (arrowheads). *Inset* in *H* shows binding of mouse IgG in the ovarian ZP in this ovary by direct immunofluorescence. (Hematoxylin and eosin, *A*, *B*, *E*, and *F*, ×100; toluidin blue stain, *C*, *D*, *G*, and *H*, ×200).

Autoantibody alone did not induce autoimmune oophoritis

We next determined the capacity of ZP Ab to induce oophoritis. Serum with a high titer of Ab to ZP was obtained from mice injected with CP1, a chimeric peptide with the native B cell epitope of pZP3 and the foreign bovine ribonuclease T epitope (RNase (94–114)). Recipients of the polyclonal Ab to $ZP3^{335–342}$ showed in vivo binding of the IgG to the ovarian ZP, and their ovaries were histologically normal (Fig. 3*A*). A more persistent and intense Ab response was elicited by active immunization with CP2, a modified CP1. In CP2, the ZP3 B cell epitope was changed from QFQIHGPR to Q*A*QIHGPR so that CP2 did not cross-react with pZP3 as a T cell epitope (Fig. 3, *C* and *D*). Hyperimmunization with CP2 in CFA induced ZP Ab response sufficient to reduce female fertility, but not to induce ovarian inflammation or disruption of normal ovarian histology (Fig. 3*B*) (17). Moreover, despite strong binding of IgG Ab to the ovarian ZP, there was no detectable complement C3 deposition in the ZP by direct immunofluorescence. Thus, a persistently high level of ZP Ab per se is not sufficient to induce oophoritis.

Cotransfer of autoantibody with T cells results in translocation of inflammation in the growing and mature follicles

The following studies, designed to induce both Ab and T cell response to ZP in the mice, directly investigated whether autoantibody, bound to the ZP, alters the distribution of T cell-mediated inflammation. In the first study, mice were immunized with CP2 in CFA to elicit an anti-ZP3 Ab response without concomitant T cell response to ZP3 (Fig. 3, *C* and *D*) (17). Two to four weeks later, the mice were challenged with $ZP3^{330–340}$, a truncated ZP3 peptide that lacks native B cell epitope, in CFA. Compared with mice injected with $ZP3^{330–340}$ in CFA alone, mice injected with both $ZP3^{330–340}$ and CP2 developed severe follicular oophoritis (Fig. 2*A*). Similar results were obtained with mice that were immunized with $ZP3^{330–340}$ in CFA, and then received mouse polyclonal mouse Ab to ZP3 2 and 7 days later (data not shown). These results further strengthen the possibility that Ab, together with T cell response, modifies distribution of ovarian inflammation. However, the $ZP3^{330–340}$ peptide, although lacking in the native B cell epitope $ZP3^{335–342}$, elicits autoantibodies to distant native B cell

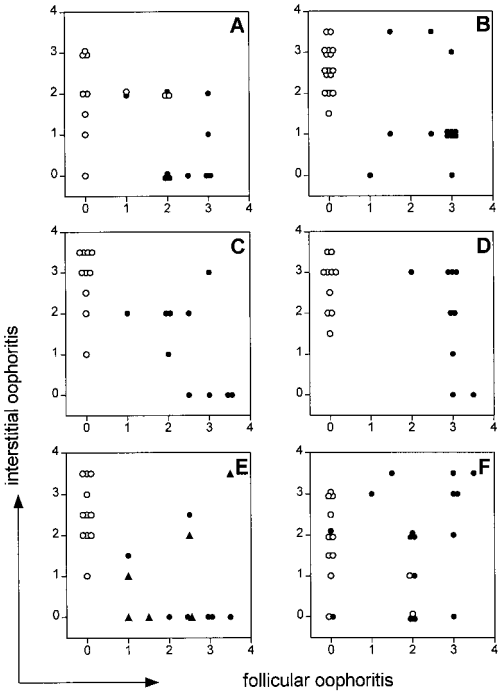

FIGURE 2. Distribution and severity of ovarian inflammation in autoimmune ovarian disease. The extent of interstitial oophoritis (vertical axis) and of follicular oophoritis (horizontal axis) is shown for individual mice, each represented by a symbol. *A*, Mice injected with the $ZP3^{330–340}$ peptide that lacks the native B cell epitope alone (○) or coinjected with CP2 (●). *B*, pZP3 T cell recipients (○) and pZP3 T cell recipients immunization with CP2 (●). *C*, pZP3 Th1 cell recipients (○) and recipients of both pZP3 Th1 cells and polyclonal mouse Ab to $ZP^{335–342}$ (●). *D*, pZP3 Th2 cell recipients (○) and recipients of both pZP3 Th2 cells and polyclonal mouse Ab to $ZP^{335–342}$ (●). *E*, pZP3 T cell recipients (○), and pZP3 T cell recipients coinjected with polyclonal mouse Ab to $ZP3^{171–180}$ (●), or with polyclonal mouse Ab to $ZP2^{121–127}$ (▲). *F*, Recipients of pZP3 T cells with rat mAb to $ZP^{335–342}$ (○) or with polyclonal rat Ab to $ZP^{335–342}$ (●).

epitopes of ZP3, through the mechanism of epitope spreading (16, 20). Because this phenomenon may confound interpretation of the results, we conducted the next two experiments.

Mice were first injected with CP2 in CFA; when their serum Ab titer reached a plateau level in 2–4 wk, they received pathogenic, ZP3-specific T cells that produced mainly IFN-γ and little IL-4 or IL-5. Compared with recipients of T cells alone, much of the ovarian inflammation in the mice that received both T cells and ZP Ab had shifted to the growing and mature follicles (Fig. 2*B*). The ovarian pathology in mice with both T cell and Ab responses, illustrated in Fig. 1, *E* and *F*, shows that many growing and mature follicles are heavily infiltrated with inflammatory cells, while the extent of interstitial oophoritis is reduced or undetectable in most

Table I. *Correlation between autoantibody to ZP3 and inflammation in mature follicles in the mice immunized with peptide ZP3 330–340*

Follicular Inflammation (severity)	Anti-ZP Ab Titer <10	50	200	>200	Total
0	98	11	10	6	125
1	2	3	9	22	36
2	0	3	12	34	49
3	0	0	5	14	19
Total	100	17	36	76	229

Table II. *Effect of CTLA4-Ig treatment in altering location of T cell-mediated inflammation*

Treatment	Anti-ZP Ab (positive/total)	Inflammation (positive/total) Interstitium	Follicle
CTLA4-Ig	0/11	7/11	0/11
L6	12/13	9/13	6/13

animals (Fig. 2*B*). The inflammatory cells in the growing mature follicles include numerous $CD4^+$ T cells (Fig. 1*G*), a few MHC II^+ macrophages (Fig. 1*H*), and no granulocytes. In contrast, mice injected with CFA and receiving the same pZP3 T cell line developed only interstitial oophoritis (Fig. 2*B*).

To completely rule out the effect of adjuvant on Ab-mediated retargeting of ovarian inflammation, mice were passively transferred with serum Ab to ZP and then with a pathogenic pZP3-specific T cell line. As shown in Fig. 2, *C* and *D*, mice that received both Ab and pathogenic T cells developed severe and frequent follicular oophoritis with minimal interstitial oophoritis. In contrast, control recipients of serum from CFA-immunized mice and pathogenic pZP3-specific T cell line had exclusively interestitial oophoritis. These results provide direct evidence that ZP Ab has retargeted the T cell-mediated oophoritis from the atretic follicles to the growing and mature ovarian follicles.

Ab specificity and the T cell cytokines did not influence Ab retargeting of T cell-mediated oophoritis

T cell retargeting was not dependent on the binding of the Ab to the B cell epitope $ZP3^{334–342}$. Abs to chimeric peptide CP3, which recognize $ZP3^{171–180}$, a native B cell epitope not cross-reactive

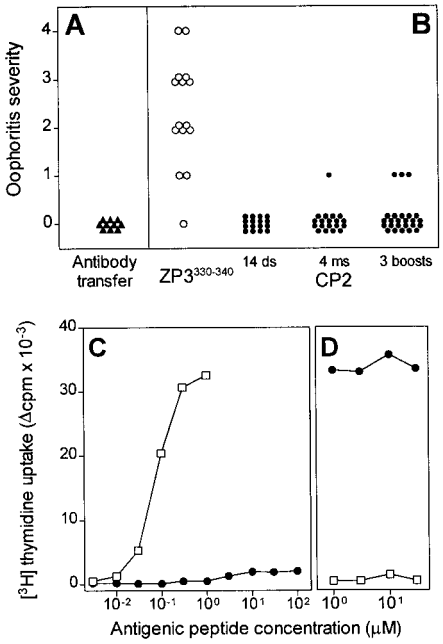

FIGURE 3. Immunologic properties of CP1 and CP2. *A*, Normal ovarian histology in recipients of Ab to $ZP3^{335–342}$ from mice immunized with CP1. *B*, Mice immunized with $ZP3^{330–340}$ that lacks the native B cell epitope $ZP3^{335–342}$ developed oophoritis (○), whereas mice immunized with CP2 by three different regimens were essentially free of oophoritis (●). *C* and *D*, In vitro T cell proliferation assay indicates the lack of cross-reaction between pZP3 and CP2: A pZP3-specific T cell line responds to pZP3 (□), but not CP2 (●) (*C*), whereas a CP2-specific T cell line responds to CP2 (●), but not pZP3 (□) (*D*).

Table III. *Prevalence and severity of ovarian atrophy in B6AF$_1$ mice with pZP3-specific T cell and or ZP3 Ab responses*[a]

Group	Induction of ZP3 or Control Ab	pZP3 T Cell Transfer (10^7 per recipient)	Total No. Mice	No. (%) of Mice with Ovarian Atrophy (0–3)			
				0	1	2	3
A	Immunization with CP2 in CFA	Yes	16	7	5	4	0
B	Transfer of ZP3 Ab	Yes	18	7	3	6	2
A + B			34	14 (41)	8 (24)	10 (29)	2 (6)
C	Immunization with CFA	Yes	16	15	1	0	0
D	Transfer of CFA Ab	Yes	10	10	0	0	0
C + D			26	25 (96)	1 (4)	0	0
E	Immunization with CP2 in CFA	No	12	12	0	0	0
F	Transfer of ZP3 Ab	No	3	3	0	0	0
E + F			15	15 (100)	0	0	0

[a] Female B6AF$_1$ mice were immunized with 50 μg of CP2 in CFA or with CFA alone, or they received 0.5 ml of hyperimmune mouse serum with ZP3 Ab. Mice in some groups also received pZP3-specific T cell line. Fifteen days after Ab and T cell transfer, ovarian histopathology was examined for evidence of atrophy, as defined in *Materials and Methods*.

with ZP3$^{335–342}$ (20), also retargeted T cells to growing and mature ovarian follicles (Fig. 2*E*). This was also true for Abs to ZP2$^{123–126}$, a B cell epitope of the ZP2 glycoprotein (Fig. 2*E*) (18). The retargeting effect was not unique to murine Abs. As shown in Fig. 2*F*, retargeting was elicited by a rat polyclonal Ab to the murine ZP3 B epitope ZP3$^{335–342}$, but interestingly, not by a rat IgG2a mAb that also recognizes the ZP3$^{335–342}$ B cell epitope (21).

We then determined whether ZP Ab uniquely retargeted ovarian inflammation induced by Th1 cells. As shown in Fig. 2, in mice with ZP-bound Ab, the ovarian inflammation transferred by both Th1 and Th2 lines was targeted to the growing and mature ovarian follicles (Fig. 2, *C* and *D*). However, in the Th2 recipients, eosinophils were the dominant inflammatory cells recruited into the growing and mature ovarian follicles.

Retargeting of autoimmune oophoritis is associated with the loss of ovarian follicles and ovarian atrophy

We next determined whether retargeting of T cell-mediated inflammation to the growing and mature ovarian follicles would lead to destruction of the functional unit of oogenesis. As shown in Table III, about 60% of mice that received both ZP Ab and pZP3 T cells exhibited significant loss of growing and mature oocytes. In contrast, only 1 of 25 T cell recipients, and none of the Ab recipients, had mild atrophy. Ovaries with severe ovarian atrophy exhibited extensive follicular destruction. Many of the oocytes were selectively destroyed, leaving behind residual inflammatory infiltrates and follicular remnants of granulosa cells without oocytes (Fig. 4, *A* and *B*).

Discussion

When autoantibody to ZP3 binds to the native determinants in the ZP glycoprotein in functional growing and mature ovarian follicles, there is a concomitant migration of leukocytes from the ovarian interstitium into the follicles, leading to destruction of the follicles. This conclusion is supported by the following observations. First, pZP3-specific, CD4 T cells primarily target the atretic follicles in the ovarian interestitial tissue, and spare the growing and mature follicles. These ovaries remained functional (3). Second, ZP3-specific Abs bind intensely to the ovarian ZP, but do not cause ovarian inflammation. Third, when both ZP3 Ab- and pZP3-specific T cells are present, the CD4 T cell-mediated ovarian inflammation is dramatically retargeted into growing and mature ovarian follicles. The phenomenon is documented for the Abs directed to several different epitopes of ZP3 and to a second ZP protein, ZP2. In addition, ZP3 Ab retargets both Th1 and Th2 dominant T cell lines and recruits their characteristic infiltrating inflammatory cells into the ovarian follicles. Significantly, the loss of ovarian follicles was associated with Ab-mediated retargeting of ovarian inflammation.

The tissue expression of the ovarian ZP Ags is strongly influenced by the process of oogenesis and the fate of the developing normal ovarian follicles. These changes, in turn, strongly influence the ovarian pathology of pZP3-specific T cell recipients. In cycling female mice, a cohort of 40–50 primordial oocytes enters the growth phase every 4–5 days. Only 10–20% of these oocytes survive and become growing and mature follicles that ovulate. These follicles are the functional units of oogenesis in the ovary. The remaining 80–90% of developing oocytes enters an apoptotic pathway and their follicles undergo atresia (22). The atretic follicles contain oocyte Ags, including the ZP. Infiltrated by occasional F4/80$^+$ macrophages that express MHC II Ags, these atretic follicles eventually disappear within the ovarian interstitium (23). Following adoptive transfer, the pZP3-specific CD4 T cells that reach the ovaries home specifically to atretic follicles, recruit leukocytes, and form multiple granulomas in the ovarian interstitium. Thus, the atretic follicles are the primary targets of pZP3-autoreactive CD4$^+$ T cells, which presumably recognize ZP3 peptide in ovarian interstitium presented by ovarian APC. In contrast, the growing and mature ovarian follicles are devoid of F4/80$^+$ or MHC class II$^+$ cells, and are rarely targeted by pZP3-specific T cells. The interstitial oophoritis in pZP3 T cell recipients can be severe and may occupy up to 80% of the interstitial space. Despite

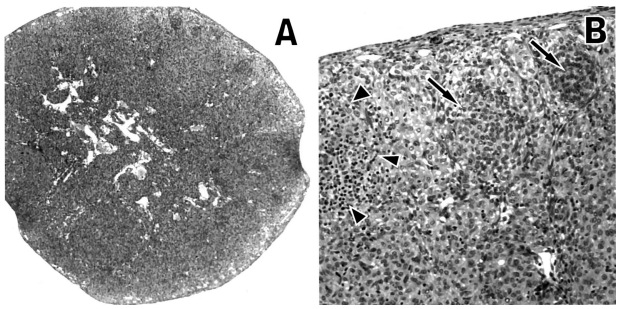

FIGURE 4. Histopathology of an atrophic ovary from the recipient of pZP3-specific Th1 line and Ab to ZP3. *A*, Severe ovarian atrophy showing loss of all growing and mature follicles. Higher magnification of the same ovary (*B*) demonstrates collapsed follicles devoid of oocytes (arrows) and residual inflammation (arrowheads). (Hematoxylin and eosin: *A*, ×50; *B*, ×200.)

intense interstitial oophoritis, these animals retain normal ovarian function, such as follicular growth and maturation, ovulation, fertilization capacity, and ability to maintain normal pregnancy (3).

On the other hand, circulating autoantibodies to ZP3 or ZP2 readily access the oocytes within the growing and mature ovarian follicles, and bind to the ZP around the oocytes. Although Ab alone may reduce fertility rate due to blockade of fertilization (17), binding of ZP Abs of different IgG subclasses to the ovarian oocytes does not lead to notable ovarian pathology in adult female mice. Specifically, ovarian inflammation and oocyte destruction are absent in adult Ab recipients. The lack of deposition of complement in the Ab-coated ZP suggests that complement activation in situ has not occurred, and this may explain the lack of tissue destruction by Ab alone**.**

However, the absence of direct Ab effect in autoimmune disease including AOD does not preclude a critical role for Ab in disease development (24–26). The most important finding of this study is the strong influence of the nonpathogenic ZP Ab on the distribution of the T cell-mediated inflammation within the ovary. When Ab binds to the ZP of the growing and mature ovarian follicles, the inflammatory cells enter the ovarian follicles, infiltrate the granulosa cell layer, penetrate the ZP, and destroy the oocytes. Although earlier studies have emphasized the cooperation between Ab and cellular immunity in autoimmune disease pathogenesis, there is little data on how T cells and Ab orchestrate tissue destruction. Autoantibodies are not required for EAE induction, but synergy between Ab and T cells in disease pathogenesis has been reported (27–33). This is particularly evident in EAE induced by the myelin oligodendrocyte glycoprotein, where demyelination occurs in the presence of encephalitogenic T cell responses and Ab response (30–34), with neutrophil infiltration (30–34). Transfer of Ab to myelin basic protein has also been shown to enhance murine EAE severity, and it was postulated that autoantibody enhanced Ag processing and presentation (27–29). Alternatively, T cell-associated inflammation may injure endothelial cells, reduce the blood-brain barrier, and facilitate entrance of demyelinating Ab to the CNS (29, 33). In collagen-induced arthritis, although Ab alone transfers synovitis to normal mice, severe and erosive arthritis occurs only when both T cells and Abs are present (35). The requirement of Ab in severe arthritis is most dramatic in the recently described spontaneous arthritis in the TCR transgenic I-A^{g7+} mice (36). A similar situation has been reported in mice with tubulointerstitial nephritis induced by immune response to a renal tubular basement membrane Ag, although the mechanism is also unclear (37, 38). Recently, it was reported that nonobese diabetic mice without B cells had periinsulitis only; thus, B cells and/or Abs are required for the pathogenesis of insulitis and diabetes (39, 40, 41). The present study has proposed a novel Ab action: an ability to focus T cell-mediated inflammation toward a tissue structure, in which the native self Ag is expressed, but is devoid of APC.

Several possible mechanisms may result in the translocation of inflammatory cells from the interstitium to the ovarian follicles. Ab to the ZP may interfere with the communication between the oocyte and the granulosa cells through the numerous intercellular gap junctions embedded in the ZP matrix, and the altered granulosa cells may signal chemotaxis of leukocytes into the ovarian follicles (42). However, simple mechanical interruption at the ZP appears unlikely because a rat IgG2a mAb, which does not activate complement (43), but binds strongly to the ZP, did not cause follicular inflammation. The Ag-Ab complexes on the ZP may activate the complement cascade to generate chemotactic peptides. However, complement component C3 is not detected, and our preliminary study indicated that retargeting occurred following serum complement depletion by the cobra venom factor, and also in C5-deficient mice (Lou, unpublished data). As a third possibility, Ab bound to the ZP may engage Fc receptors on cells adjacent to the ZP leading to **a** chemotactic signal (44).

It should be emphasized that Ab mechanism, and other mechanisms that have been proposed for retargeting of autoimmune inflammation (4–13), are not mutually exclusive. Indeed, it is our hypothesis that Ab retargeting may require activation of Th cells and macrophages, as well as up-regulation of MHC class II and altered tissue migration of APC. Regardless of how Ab to the ZP mediates leukocyte chemotaxis, the result of this study has made it clear that Ab has the capacity to recruit a wide range of inflammatory cells into the growing and mature ovarian follicles. If the initial chemoattractant was aimed at the T cells, then the factor(s) could attract both Th1 and Th2 cells. Alternatively, Ab binding may translocate any leukocytes that have entered the ovarian interstitial space from blood, including the Th1-recruited monocytes/macrophages and the Th2-recruited eosinophils. In summary, the present study has demonstrated that the interaction between Ab and T cells, within a target organ, can dictate the distribution of autoimmune inflammation, and hence determine the clinical outcome of an autoimmune disease.

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