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S H Rhim, … , J Dean, K S Tung

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Research Article

We describe a novel experimental system in mice for the study of ovarian autoimmune disease, a condition encountered in women with premature ovarian failure. The ovarian autoimmune disease is induced in B6AF1 mice by a 15-amino acid peptide (Cys-Ser-Asn-Ser-Ser-Ser-Ser-Gln-Phe-Gln-Ile-His-Gly-Pro-Arg) from mouse ZP3, the sperm-binding component of the zona pellucida that surrounds growing and mature oocytes. Whereas the peptide induces both T cell and antibody responses, adoptive transfer of CD4+ T cell lines derived from affected animals causes oophoritis without observable antibodies to the zona pellucida peptide. The primacy of the T cell response in the pathogenesis of disease is further substantiated by defining oophoritogenic peptides as small as eight amino acids (Asn-Ser-Ser-Ser-Ser-Gln-Phe-Gln) that do not elicit an antibody response to the full-length ZP3 peptide. The identification of a well characterized peptide as a causative agent of autoimmune oophoritis should facilitate understanding of the pathogenesis of this T cell-mediated autoimmune disease. Because the proteins of the zona pellucida are conserved among mammals (the mouse and human ZP3 proteins are 67% identical), this murine model may lead to better understanding of the pathogenesis of human autoimmune oophoritis.

Autoimmune Disease of the Ovary Induced by a ZP3 Peptide from the Mouse Zona Pellucida

Sung Hee Rhim, Sarah E. Millar,* Frank Robey,‡ An-Ming Luo, Ya-Huan Lou, Terecita Yule, Paul Allen, Jurrien Dean,* and Kenneth S. K. Tung

*Departments of Pathology and Medicine, Division of Laboratory Medicine, Washington University School of Medicine, St. Louis, Missouri 63110; *Laboratory of Cellular and Developmental Biology, National Institute of Digestive Diseases and Kidney, and ‡Laboratory of Cellular Development and Oncology, National Institute of Dental Research, National Institutes of Health, Bethesda, Maryland 20892*

Abstract

We describe a novel experimental system in mice for the study of ovarian autoimmune disease, a condition encountered in women with premature ovarian failure. The ovarian autoimmune disease is induced in $B6AF_1$ mice by a 15-amino acid peptide (Cys-Ser-Asn-Ser-Ser-Ser-Ser-Gln-Phe-Gln-Ile-His-Gly-Pro-Arg) from mouse ZP3, the sperm-binding component of the zona pellucida that surrounds growing and mature oocytes. Whereas the peptide induces both T cell and antibody responses, adoptive transfer of $CD4^+$ T cell lines derived from affected animals causes oophoritis without observable antibodies to the zona pellucida peptide. The primacy of the T cell response in the pathogenesis of disease is further substantiated by defining oophoritogenic peptides as small as eight amino acids (Asn-Ser-Ser-Ser-Ser-Gln-Phe-Gln) that do not elicit an antibody response to the full-length ZP3 peptide. The identification of a well characterized peptide as a causative agent of autoimmune oophoritis should facilitate understanding of the pathogenesis of this T cell–mediated autoimmune disease. Because the proteins of the zona pellucida are conserved among mammals (the mouse and human ZP3 proteins are 67% identical), this murine model may lead to better understanding of the pathogenesis of human autoimmune oophoritis. (*J. Clin. Invest.* 1992. 89:28–35.) Key words: oophoritis • premature ovarian failure • T cell–mediated disease • zona pellucida • ZP3

Introduction

Human autoimmune oophoritis is a pathologic entity encountered in women with premature ovarian failure (1, 2), the polyglandular autoimmunity syndrome, (3) and cystic ovarian disease (4). A comparable disease, murine autoimmune oophoritis, has been described in certain strains of female mice [$(C57BL/6 \times A/J)F_1$, $(SWR/J \times A/J)F_1$, BALB/cBy], thymectomized between 1 and 4 d after birth. Initial inflammatory infiltrations in the ovary culminate in intense oophoritis between 5 and 14 wk and eventually the loss of ova and the collapse of ovarian follicles results in grossly atrophic ovaries. Animals with sham thymectomies do not develop disease (5–7). Since neonatal thymectomy of $(C57B1/6 \times A/J)F_1$ results in the maintenance of at least one T cell receptor ($V_\beta 11^+$) that would otherwise be clonally deleted in the adult thymus, it raises the possibility that the oophoritis may occur because of enrichment of self-reactive T cells from the neonatal repertoire (8).

Investigations into the pathogenesis of autoimmune oophoritis has been hampered by the lack of an identifiable initiating antigen(s). We have sought to identify potential target antigens in the ovary in order to establish an experimental system to study the pathogenesis of murine autoimmune oophoritis in thymus intact animals. One source for such antigens is the zona pellucida, an extracellular matrix surrounding growing oocytes that is composed of three sulfated glycoproteins, ZP1, ZP2, and ZP3 (9, 10). ZP3 (83 kD) acts as the primary sperm receptor, mediating sperm binding to ovulated eggs and inducing the sperm acrosome reaction at the time of fertilization (11). The single-copy *Zp*-3 gene (12, 13) is transcribed uniquely in growing oocytes (14, 15) and the resultant protein can be detected in ovaries within 3 d of birth. The 424-amino acid sequence of ZP3 has been deduced from a full-length cDNA (16) and is 67% identical to the recently described human ZP3 protein (17).

In this paper we describe the induction of ovarian autoimmune disease in $B6AF_1$ mice with a 15-amino acid oophoritogenic peptide, $ZP3^{328-342}$. In transfer studies to naive animals we demonstrate the pathogenetic role of T cells in eliciting disease and, using truncated peptides, define an eight-amino acid peptide as a minimal oophoritogenic peptide.

Methods

Animals. $(C57BL/6 \times A/J)F_1$ ($B6AF_1$), A/J, C57BL/6 (B6), and BALB/cBy adult female mice were obtained from the Jackson Laboratory, Bar Harbor, ME, or from Washington University School of Medicine. Random bred NIH Swiss mice were housed at the National Institutes of Health. Mice were studied at 6–9 wk of age.

Peptides. ZP3 peptides were synthesized by solid-phase synthesis and purified by high pressure liquid chromatography on a C18 reverse-phase column. Peptide composition was verified by amino acid analysis. The results of studies based on peptide $ZP3^{328-342}$ (CSNSSSSQFQIHGPR, mol wt 1,615) and peptide $ZP3^{328-343}$ (CSNSSSSQFQIHGPRQ, mol wt 1,743) were indistinguishable and the two peptides were used interchangeably in the study. Additional truncated 328–342 peptides are listed in Fig. 1. As controls, mice were immunized with mouse ZP3 peptides encompassing residues 183–196 (peptide A, EKSAPTFHLGEVAH), residues 201–221 (peptide B, HLPLQLFVDHCVATPS), and residues 371–398 (peptide C, KANDQTVEGWTASAQTS). Mycobacterial antigen was prepared by suspending 200 mg of lyophilized *Mycobacterium tuberculosis* organisms (H37Ra, Difco Lab-

Address reprint requests to Dr. Tung, Department of Pathology, University of Virginia, Charlottesville, VA 22908.

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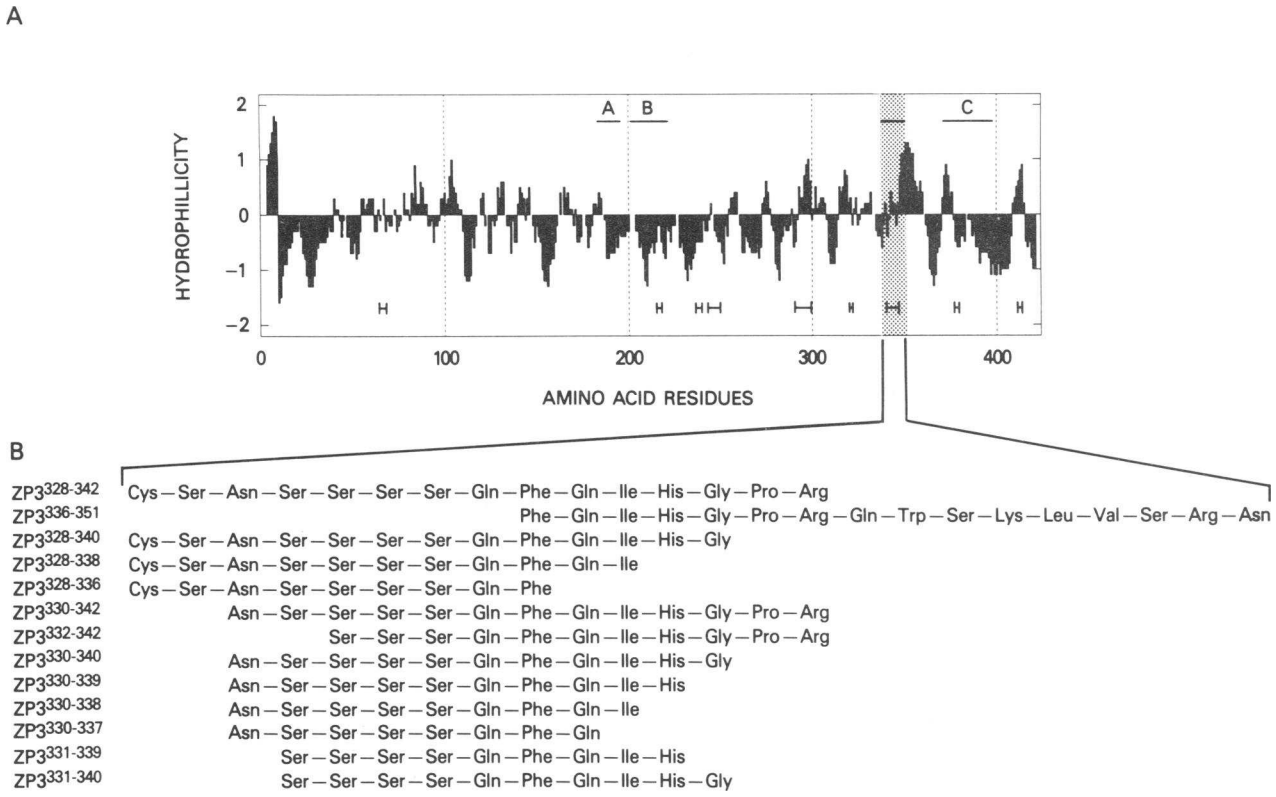

Figure 1. Mouse ZP3 peptides. (*A*) Hydrophilicity plot (26) of the 424 amino acid mouse ZP3 protein. The positions of peptide A (residues 183–196), peptide B (amino acids 206–221) and peptide C (amino acids 371–398) are indicated at the top. Shaded region represents position of truncated peptides indicated in *B*. (*B*) Amino acid sequence of 13 ZP3 peptides positioned between residues 328 and 351 used in study.

oratories, Inc., Detroit, MI) in 2 ml of PBS. The solution was sonicated and clarified by microfugation.

Immunization. The appropriate dose of peptide dissolved in deionized/distilled water, was emulsified in an equal volume of complete Freund's adjuvant containing 4.5 mg/ml *Mycobacterium tuberculosis* or incomplete Freund's adjuvant. Each mouse received 0.1 ml of the antigen and adjuvant emulsion in the two hind footpads. Some mice also received intraperitoneally 5 μg of an extract of *Bordetella pertussis* enriched in pertussigen (gift of Dr. John Munoz, National Institute of Allergy and Infectious Disease, Hamilton, MT) (18).

Histologic assessment of ovarian disease. Ovaries were fixed for 24 h in Bouin's fixative and embedded in paraffin. Approximately 50 serial step sections (5 μm) stained with hematoxylin and eosin were examined. Histopathology of ovaries, as coded specimens, were evaluated by an independent observer. Ovarian pathology was graded with increasing severity from 1 to 4 (1, focal inflammation in interstitial space; 2 and 3, increasing multifocal inflammatory foci and/or granuloma between and within ovarian follicles; 4, loss of ovarian follicles and ovarian atrophy).

Antigen-specific lymphocyte proliferation assay. Popliteal and inguinal lymph nodes were dispersed into single cell suspension in RPMI-1640 medium supplemented with 1% sodium pyruvate, 1% nonessential amino acids, 1% 200 mM glutamine, 100 U of penicillin, 100 μg/ml streptomycin, 5×10^{-5} M 2-mercaptoethanol, and 10% heat-inactivated fetal calf serum (Fisher Scientific Co., Pittsburgh, PA) (complete medium). Cells were cultured in flat-bottom microtiter plates (Costar, Cambridge, MA) at 3×10^5 cells per well in a volume of 0.2 ml in the presence of varying concentrations of filter-sterilized ZP3 peptides or mycobacterial antigens. Mycobacterial antigen was diluted 1:100 in complete medium and 50 μl were used per well. After 4 d in 37°C in 5% CO_2 and air, 0.5 μCi of [^{3}H]thymidine was added and the amount of cell-associated thymidine determined 8 h later. Data were expressed as delta counts per min (experimental counts per minute minus background counts per minute) and as a stimulation index (experimental counts per minute divided by background counts per minute). Background was calculated from cells not stimulated with peptide.

Production of T cell lines. B6AF$_1$ female mice were immunized with 100 μg of $ZP3^{328-340}$ or $ZP3^{330-342}$ in complete Freund's adjuvant. 14 d later, their lymph node cells were isolated and stimulated in vitro with 30 μM 328–342 in complete medium. 4 d later, viable lymphocytes were obtained by centrifugation on a Hypaque-Ficoll gradient and dispersed in complete medium with 1–2% of lymphokine-enriched supernatant of EL4 cell culture containing 1.5 U of interleukin 2 activity. After 7–10 d, the lymphocytes were restimulated with 30 μM of $ZP3^{328-342}$ in complete medium and rested, as described above. After four cycles of alternating peptide stimulation and resting period, two stable cell lines of $CD4^+CD8^-$ phenotype were obtained that responded specifically to $ZP3^{328-342}$.

Adoptive transfer of oophoritis. B6AF$_1$ female mice were immunized with 50 μM of $ZP3^{328-342}$ in complete Freund's adjuvant in the hind footpads. 10 d later, the inguinal, popliteal, subscapular, and paraaortic lymph nodes were dissected and from them viable lymph node cells were obtained. The cells were stimulated at 5×10^6/ml in complete medium in the presence of concanavalin A (3 μg/ml) for 48 h. Viable cells containing the activated T cell blasts were harvested by centrifugation on a Hypaque-Ficoll gradient, washed, and transferred intraperitoneally into untreated adult B6AF$_1$ female recipients. T cell lines having just been stimulated by $ZP3^{328-342}$ were similarly transferred into untreated adult B6AF$_1$ female recipients. Ovaries of cell recipients were studied histologically 14 d later.

Detection of serum antibody to peptide $ZP3^{328-342}$. Each well of a 96-well flat bottom plate (Costar ELISA plate) was coated by 100 μl of a 10 μM $ZP3^{328-342}$ peptide solution and blocked by 2% bovine serum albumin in phosphate-buffered saline (PBS). Test serum triplicates at 1:50 and 1:200 dilutions were added and incubated for 60 min at 20°C.

The plates were washed six times with PBS and 0.05% Tween 20, and incubated with goat anti-mouse immunoglobulins labeled with peroxidase (Amersham Corp., Arlington Heights, IL) (1:1,000) for 60 min at 20°C. After the plates were washed thoroughly, *O*-phenylenediamine and hydrogen peroxide were added. Enzyme reaction was stopped by 2.5 N sulfuric acid after 15–30 min, and the absorbance at 490 nm was determined on an ELISA reader. A standard curve was constructed with serial dilutions of a pooled hyperimmune antiserum against $ZP3^{328-342}$ and the experimental values expressed as the percent of the midpoint of the standard curve.

Direct immunofluorescence. Ovaries were snap frozen in liquid nitrogen and embedded in OCT compound, and 5-μm-thick frozen sections were cut in a cryostat. After the tissue sections were fixed in 90% ethanol for 15 min, they were rinsed in PBS and incubated with fluorescein isothiocyanate–conjugated goat antiserum IgG containing antibody to mouse immunoglobulins (IgG, IgA, or IgM), mouse IgGFc, or mouse IgM (Southern Biotechnology, Birmingham, AL) for 30 min. After the sections were rinsed in PBS followed by glycerol containing 10% PBS, a coverslip was applied and the slides were examined and photographed with a Zeiss fluorescence microscope.

Results

Induction of murine autoimmune oophoritis by $ZP3^{328-342}$. Four ZP3 peptides (amino acids 183–196, 206–221, 336–351, 371–398), selected for their capacity to form α-helical structures, were tested for their ability to induce oophoritis (Fig. 1 *A*). None elicited disease. However, a fifth peptide (amino acids 328–343) was a potent inducer of oophoritis in $B6AF_1$, $(C57BL/6 \times A/J)F_1$, mice (Table I, expt. 1). This peptide ($ZP3^{328-342}$) contains the seven-amino acid binding site of an anti-ZP3 monoclonal antibody that causes infertility in female mice (19). When coupled to keyhole limpet hemocyanin, $ZP3^{328-343}$ produces infertility in NIH Swiss mice without detectable ovarian disease (20).

In contrast, when $B6AF_1$ mice were immunized with 50 nmol (85 μg) of $ZP3^{328-342}$ in complete Freund's adjuvant, 48 of 56 (86%) developed oophoritis after a single injection (Table I, expt. 1). Similar results were obtained with $ZP3^{328-343}$ and the two peptides were used interchangeably as noted. The disease began on day 6 with mild focal infiltrations of lymphoid cells confined to the interstitial spaces. By 10 d the disease prevalence was 85% (Table I, expt. 2) and granulomatous nodules had developed in the interstitium with a central cluster of macrophages surrounded by lymphocytes. Later in the disease, ovarian follicles were infiltrated by inflammatory cells (lymphocytes, macrophages, neutrophils, multinucleated giant cells) and the largest follicles (antral) had inflammatory cells among granulosa cells, adjacent to the zona pellucida and inside the oocytes. In contrast, primary follicles and luteal follicles were free of inflammation. By 21 d, in ovaries with severe disease, most of the large ovarian follicles were lost and the ovaries had become atrophic (Fig. 2).

Moderate disease was found in three of three animals immunized with 10 nmol (17.5 μg) of $ZP3^{328-342}$ peptide and only mild ovarian pathology was found in two of three mice immunized with 2 nmol (3.5 μg) of the peptide (Table I, expt. 1). In all cases the pathology of immunized mice was confined to the ovaries. Disease of comparable frequency and severity was elicited by the ZP3 peptide emulsified in complete- or incomplete Freund's adjuvant and lymph nodes cells derived from animals in each group proliferated in response to $ZP3^{328-342}$ (see below). Injection of pertussis toxin as an additional adjuvant did not significantly alter the severity or prevalence of the ovarian disease (data not shown).

In addition to $B6AF_1$ mice, BALB/cBy female mice immunized with $ZP3^{328-342}$ in complete Freund's adjuvant developed autoimmune oophoritis. However, when the parental strains of $B6AF_1$ were immunized with the same peptide, A/J females, but not C57BL/6 females, developed oophoritis which suggests

Table I. Induction of Murine Autoimmune Oophoritis with ZP3 Peptides

Exp	Mouse	ZP3 peptide in CFA	Antigen dose	Day	Oophoritis—incidence and graded severity				
					Incidence	1	2	3	4
			nmol						
1	$B6AF_1$	328–342	50	10	48/56	20	14	11	3
			10	10	3/3	0	3	0	0
			2	10	2/3	2	0	0	0
	$B6AF_1$	183–196	50	10	0/4	—	—	—	—
		206–221	50	10	0/4	—	—	—	—
		371–398	50	10	0/4	—	—	—	—
		336–351	50	10	0/8	—	—	—	—
	$B6AF_1$	CFA	0	10	0/20	—	—	—	—
2	$B6AF_1$	328–342	50	2–3	0/7	—	—	—	—
				6	2/5	2	0	0	0
				10	13/14	2	5	6	0
				16	4/4	0	0	4	0
				21	4/4	0	0	0	4
3	BALB/cBy	328–342	50	10	1/2	0	1	0	0
	C57BL/6		50	10	0/4	—	—	—	—
	A/J		50	10	3/5	1	2	0	0
	NIH Swiss	328–342	50	10	0/5	—	—	—	—
	NIH Swiss		50	29	1/5	1	0	0	0

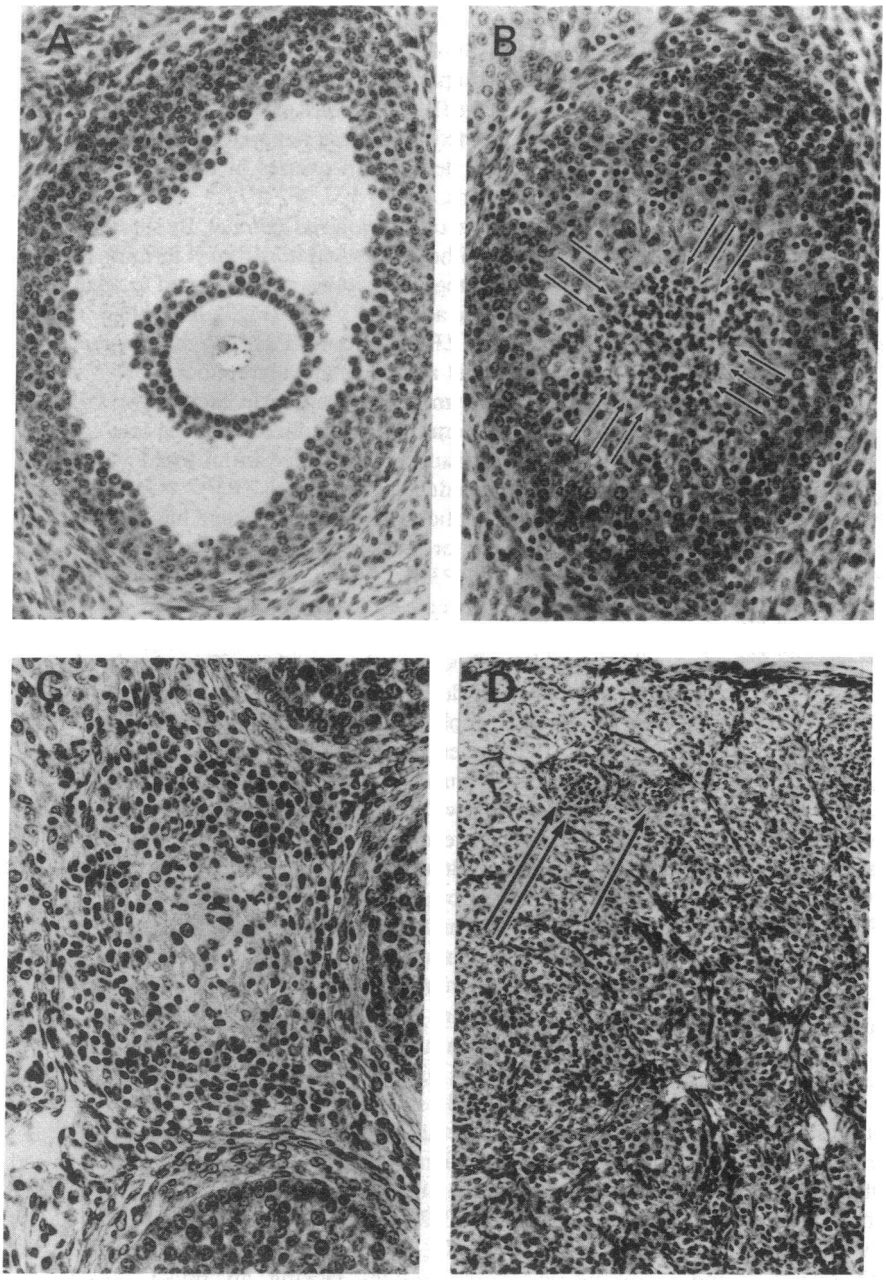

Figure 2. Histopathology of murine autoimmune oophoritis. (*A*) Normal murine Graafian antral follicle. (*B*) severe oophoritis with inflammatory cells invading Graafian follicles in mouse 10 d after immunization with 50 nmol of $ZP3^{328-342}$. Note lymphocyte infiltration in the granulosa cell layer and accumulation of neutrophils and macrophages in the region of the oocyte (*arrows*). (*C*) Granuloma in interfollicular area of $B6AF_1$ mouse ovary after immunization with $ZP3^{328-342}$. (*D*) 21 d after immunization with $ZP3^{328-342}$. There is loss of large follicles and ovarian atrophy. Arrows point to small ovarian follicles without oocytes. Sections stained with hematoxylin and eosin (*A–C*, ×400; *D*, ×50).

that susceptibility to disease is a dominant genetic trait that is not present in all strains of mice (Table I, expt. 4). Likewise, only 1/10 NIH Swiss mice had mild disease (at 29 d) after immunization with $ZP3^{328-342}$ (Table I, expt. 3) in accord with the lack of pathology noted after immunization of $ZP3^{328-343}$ coupled to keyhole limpet hemocyanin (20).

Immune response to $ZP3^{328-342}$. Lymphocytes, isolated from female $B6AF_1$ mice immunized with $ZP3^{328-342}$ in complete Freund's adjuvant, proliferated in response to $ZP3^{328-342}$ but not to two control ZP3 peptides (Fig. 3). The earliest response was detected 3 d after immunization, several days before the onset of oophoritis, and coincided with the earliest lymphocyte proliferative response to mycobacterial antigen, a component of complete Freund's adjuvant. Lymphocytes from female $B6AF_1$ immunized with $ZP3^{328-342}$ in incomplete Freund's adjuvant also proliferated in response to $ZP3^{328-342}$ (data not shown). Serum antibodies to solubilized $ZP3^{328-342}$ peptide were detected 10 d after immunization and were present in $B6AF_1$ female mice immunized with 50 nmol of $ZP3^{328-342}$ in complete Freund's adjuvant (Table II). In addition to serum antibodies, IgG (but not IgM) binding to intraovarian zonae pellucidae was detected in ovaries of mice immunized with $ZP3^{328-342}$ in complete Freund's adjuvant (Fig. 4 *A*) or incomplete Freund's adjuvant (Fig. 4 *C*).

Adoptive transfer of murine autoimmune oophoritis. To investigate the role of T cells in murine autoimmune oophoritis, lymph node cells from $B6AF_1$ female mice immunized with $ZP3^{328-342}$ in complete Freund's adjuvant were stimulated in vitro with the T cell mitogen concanavalin A and transferred into normal syngeneic adult female recipients. 14 d later, multi-

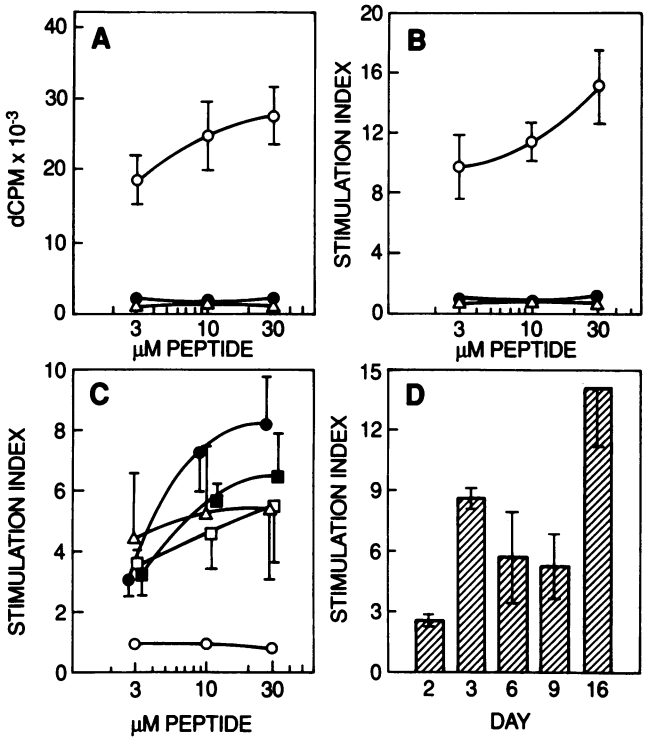

Figure 3. Specificity and kinetics of lymphocyte proliferative response. (*A*) [^{3}H]Thymidine incorporation (dCPM) into lymph node cells derived from B6AF$_1$ mice immunization with ZP3$^{328-342}$ in complete Freund's adjuvant: (○) after stimulation with ZP3$^{328-342}$; (●) with ZP3$^{202-221}$; and (△) with ZP3$^{183-196}$. (*B*) Same as *A* but with data expressed as stimulation index (experimental data divided by background). (*C*) ZP3$^{328-342}$ stimulation of lymph nodes cells obtained at: (○), day 2; (●), day 3; (□), day 6; (△) day 9; (■) day 16 after immunization of B6AF$_1$ mice with ZP3$^{328-342}$ in complete Freund's adjuvant. (*D*) Mean stimulation to mycobacterial antigen of lymph node cells derived as in *C*. Data expressed as mean and standard error of 23 mice (*A* and *B*) and 6 mice (*C* and *D*) per data point.

ple granulomas and heavy lymphocytic infiltrates were noted in ovaries of all recipients (Fig. 5 *A*, Table II). In contrast, recipients of concanavalin A–activated lymph node cells from adjuvant alone immunized donors were normal.

Two CD4$^+$ T cell lines (ZP3A and ZP3B) were derived from B6AF$_1$ female mice immunized either with ZP3$^{328-340}$ and ZP3$^{330-342}$, respectively, by repeated stimulation of lymph node cells with ZP3$^{328-342}$ and IL-2. After stimulation by ZP3$^{328-342}$ or concanavalin A in culture, ~ 1.1–1.5 × 10^7 cells were transferred to normal B6AF$_1$ female recipients. Recipients developed oophoritis comparable in severity to those of the cell donors (Fig. 5 *B*, Table II). Antibodies against the ZP3$^{328-342}$ peptide were not detected in the sera nor were antibodies against the zona pellucida detected in ovaries of the recipients that developed oophoritis.

Mapping smaller oophoritogenic epitopes. By studying peptides truncated at either the amino or carboxyl terminus of the ZP3$^{328-342}$, oophoritogenic epitopes were mapped to regions as small as eight amino acids, ZP3$^{330-337}$ (Fig. 1 *B*, Table III). Two truncated peptides, ZP3$^{328-336}$ and ZP3$^{331-339}$, did not elicit disease in mice and did not stimulate a lymphocyte proliferative response in either lymph node cells or in each of two CD4$^+$ T cell lines derived from mice immunized with the original peptide ZP3$^{328-342}$. As anticipated, mice immunized with these peptides did not produce antibodies to ZP3$^{328-342}$. In these two non-pathogenic peptides, a single amino acid has been deleted from the amino or carboxyl boundary of the peptide, ZP3$^{330-337}$. Thus, ZP3$^{330-337}$ likely represents the most potent minimal oophoritogenic epitope within ZP3$^{328-342}$.

There were eight truncated peptides that caused disease but did not elicit antibodies to the peptide in affected animals (Table III). The incidence and severity of disease elicited by the truncated oophoritogenic peptides varied from mild (ZP3$^{332-342}$) to severe (e.g., ZP3$^{330-342}$, ZP3$^{330-340}$). Seven of these peptides stimulated a lymphocyte proliferative response in vitro in each of two T cell lines derived from affected animals (although only five elicited an positive response in lymph node cells derived from B6AF$_1$ immunized with ZP3$^{328-342}$, Table II). Only the smallest oophoritogenic peptide, ZP3$^{330-337}$, did not stimulate either lymph node cells or T cell lines to proliferate (Table III). The reason for this is not clear, but may be related to differences in presentation of the epitope in vivo (to elicit disease) and in vitro (to assay for cell proliferation). Taken together, these data suggest that there are several epitopes between amino acids 330 and 340 that can cause disease. The precise amino acid residues in ZP3$^{328-342}$ critical for disease induction are currently under investigation.

High antibody titers were detected only in mice immunized with peptides containing the monoclonal antibody binding site (amino acids 336–342) and at least six additional amino acids which provided a T_{Helper} epitope (Table III). In contrast, mice immunized with peptides lacking an intact monoclonal antibody binding site produced no or minimal antibody to ZP3$^{328-342}$ (Table III). Thus, the region 336–342 must be the

Table II. Induction of Murine Autoimmune Oophoritis by Adoptive Transfer

Cell transferred	Donor immunization	In vitro treatment	Injected cells	Oophoritis—incidence and graded severity				
				Incidence	1	2	3	4
Lymph node cells	ZP3$^{328-342}$	Con A	5 × 10^7	9/9	2	2	4	1
Lymph node cells	CFA	Con A	5 × 10^7	0/7	—	—	—	—
T cell Line ZP3A	ZP3$^{328-340}$/CFA	ZP3$^{328-342}$	1.5 × 10^7	2/2	0	1	1	0
		Con A	1.5 × 10^7	4/4	1	1	2	0
T cell line ZP3B	ZP3$^{330-342}$/CFA	ZP3$^{328-342}$	1.1 × 10^7	4/5	1	3	0	0
		Con A	1.5 × 10^7	4/4	1	1	2	0

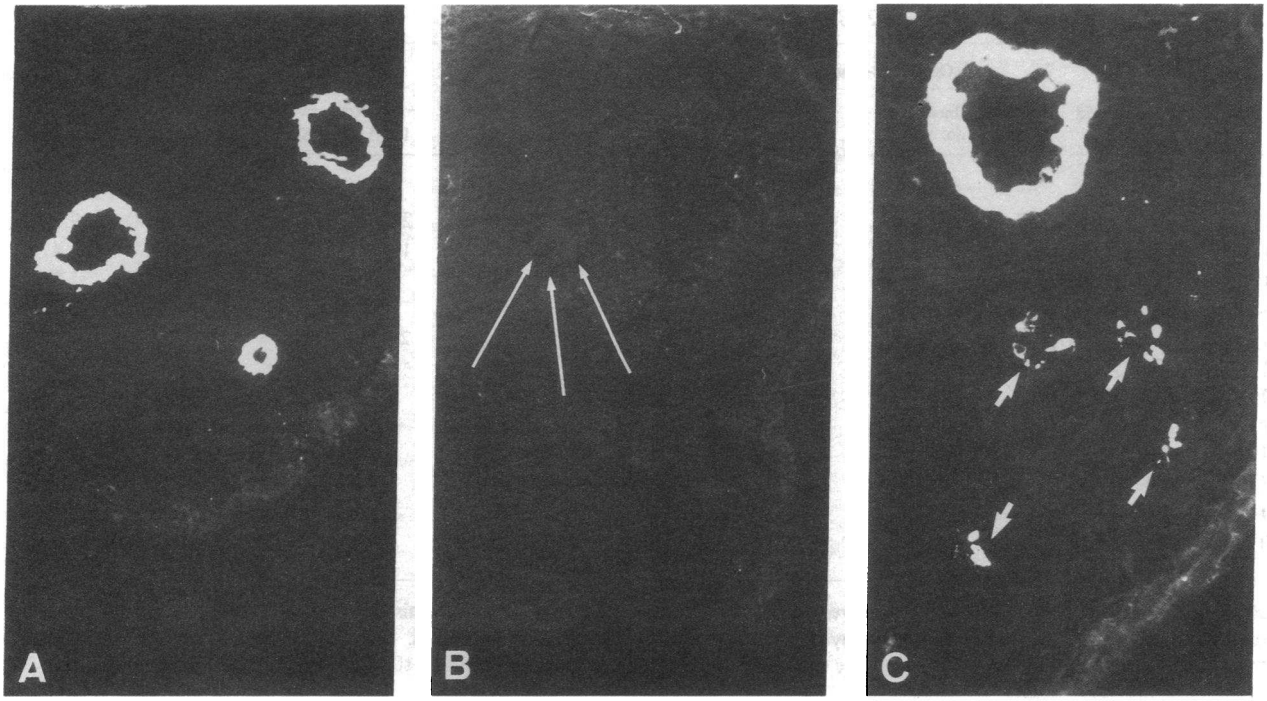

Figure 4. Immunofluorescence of $B6AF_1$ mouse ovaries. (*A*) Ovarian section stained with fluorescein-conjugated antibody against IgG detects IgG bound to zona pellucida in mouse immunized with peptide $ZP3^{328-342}$ in complete Freund's adjuvant. (*B*) Same as *A* except that control mouse was immunized complete Freund's adjuvant alone. Arrows indicate unstained zona pellucida. (*C*) Same as *A* but after immunization with $ZP3^{328-342}$ in incomplete Freund's adjuvant. Arrows in *C* in point to fragmented zonae pellucidae in atretic follicles (*A*, ×100; *B* and *C* ×200).

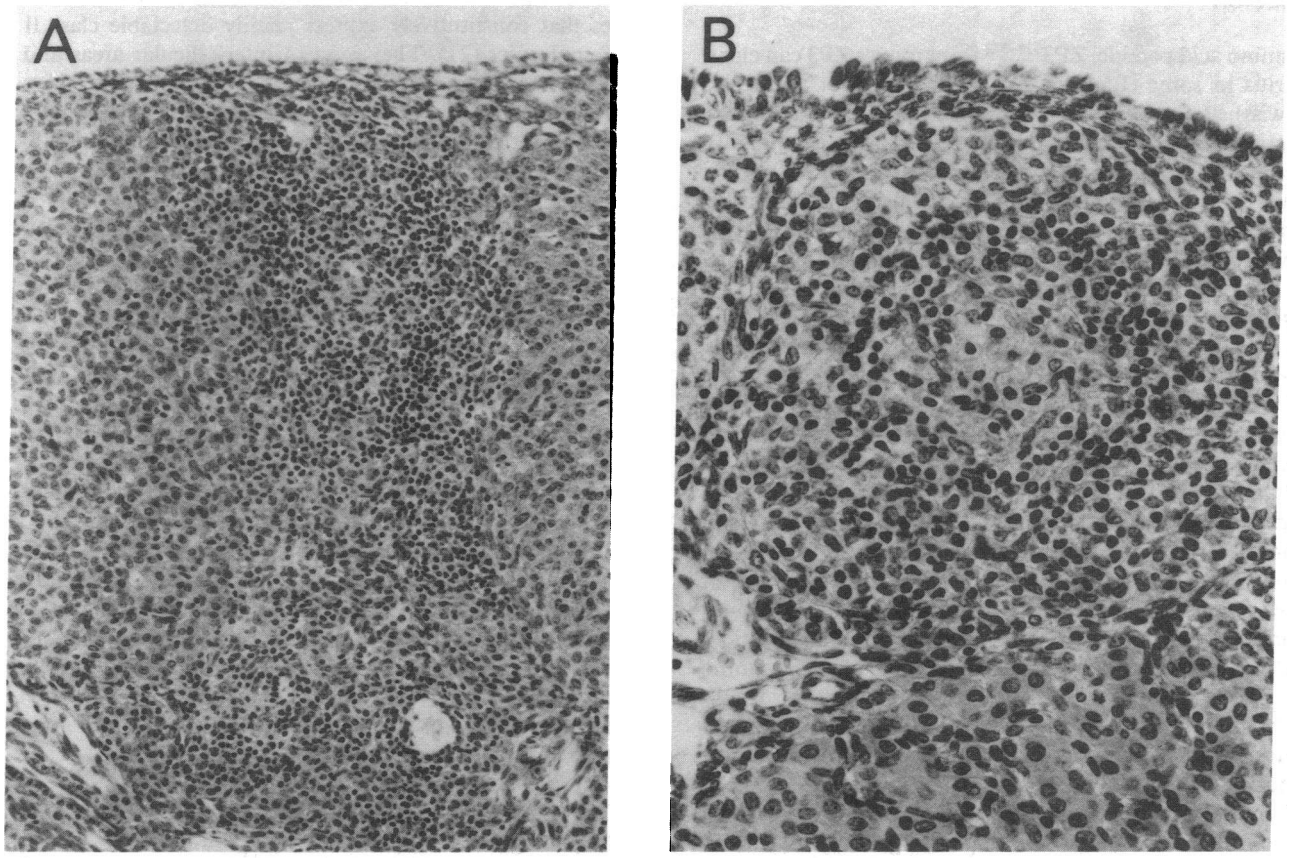

Figure 5. Oophoritis in the recipients of lymphocyte transfers from affected $B6AF_1$ mice. (*A*) Ovarian section of $B6AF_1$ mouse 14 d after injection of 5×10^7 concanavalin A–activated lymph node cells from $B6AF_1$ mice with oophoritis. Note heavy infiltration of lymphocytes in interstitial space. (*B*) same as *A* after injection of 1.5×10^7 cells of ZP3A T cell line derived from an affected $B6AF_1$ mouse. Note granulomatous inflammation in interfollicular space. Sections stained with hematoxylin and eosin (*A*, ×100; *B*, ×200).

Table III. Immune Response of B6AF$_1$ Mice to ZP3$^{328-342}$ and Truncated Derivatives

Position in ZP3	Amino acid sequence	Oophoritis—incidence and graded severity					Antibody titer* to ZP3$^{328-342}$	T cell response		
		Incidence (no. of mice)	1	2	3	4	ELISA (SE)	LPR‡ mean (SE)	Line ZP3A§	Line ZP3B§
		%							*dpm*	
328–342	CSNSSSSQFQIHGPR	86 (56)	20	14	11	3	133.4 (19.9)	25,442 (5,958)	135,020	59,336
328–340	CSNSSSSQFQIHG	55 (20)	5	4	1	1	1.5 (0.3)	14,504 (3,265)	45,797	5,474
328–338	CSNSSSSQFQI	43 (28)	3	7	2	0	2.1 (0.8)	−345 (358)	38,046	31,640
328–336	CSNSSSSQF	0 (9)	—	—	—	—	1.1 (0.3)	−29 (108)	323	313
330–342	NSSSSQFQIHGPR	97 (20)	4	8	10	6	31.0 (7.7)	29,438 (5,087)	108,593	53,760
332–342	SSSQFQIHGPR	14 (14)	2	0	0	0	0.8 (0.1)	7,242 (2,297)	10,280	3,597
330–340	NSSSSQFQIHG	92 (13)	3	6	0	3	3.9 (1.8)	6,784 (1,936)	106,043	60,776
330–339	NSSSSQFQIH	77 (13)	1	3	4	2	2.1 (0.8)	3,589 (246)	31,877	34,973
330–338	NSSSSQFQI	56 (16)	2	3	3	1	1.3 (0.3)	−649 (361)	15,977	1,096
330–337	NSSSSQFQ	57 (21)	4	5	3	0	0.9 (0.1)	508 (230)	300	280
331–339	SSSSQFQIHG	72 (18)	0	6	5	2	1.1 (0.1)	2,104 (340)	37,007	19,127
331–340	SSSSQFQIH	0 (18)	—	—	—	—	1.2 (0.2)	954 (561)	223	330

* Antibody titer detected by ELISA to ZP3$^{328-342}$ expressed as mean percent binding of standard antiserum to ZP3$^{328-342}$. ‡ dCPM of [^{3}H]thymidine incorporation in lymphocyte proliferation assay (LPR) in mice immunized with ZP3 peptides. § Mean dCPM of three determinations of [^{3}H]thymidine (dpm) incorporated in the T cell line (ZP3A and ZP3B) after stimulation with 30–100-μm peptide.

main, if not the only B cell epitope of 328–342, and antibody response to the epitope is not required for induction of autoimmune oophoritis.

Discussion

A 15-amino acid peptide, ZP3$^{328-342}$ from mouse ZP3 can elicit oophoritis in some (B6AF$_1$, BALB/cBy, A/J) but not other (B6, Swiss) strains of mice. A novel feature of the oophoritogenic peptide is that it contains both a T cell epitope and a B cell epitope (20). Thus, both T cell and antibody responses are potentially important mechanisms in pathogenesis of murine autoimmune oophoritis. However, adoptive transfer of disease by lymph node cells and two CD4$^+$ T cell lines derived from mice immunized with peptides ZP3$^{330-342}$ and ZP3$^{328-340}$ strongly suggests that the primary mechanism of the disease is T cell mediated. Whether lymph node cells from male mice immunized with ZP3 peptide also transfer oophoritis is under study. The absence of circulating antibodies to the peptides in the T cell recipients indicates that disease can occur without the involvement of antibodies.

That a T cell response is sufficient for induction of murine autoimmune oophoritis was further substantiated by the study of truncated ZP3$^{328-342}$ peptides. Seven peptides that lack the antibody binding site previously defined by responses of outbred Swiss mice, elicit severe oophoritis without concomitant antibody response to ZP3$^{328-342}$ (Table II). These peptides include a minimal oophoritogenic peptide of eight amino acids, ZP3$^{330-337}$, which overlaps the seven-amino acid antibody binding site (ZP3$^{336-342}$) by two residues. Although a T cell mechanism alone is sufficient and antibody is not required for murine oophoritis, the present study has not determined whether antibody alone can also induce disease. The truncated peptides that do not contain the oophoritogenic epitope afford useful reagents with which to resolve this issue.

The study also begins to elucidate the nature of ZP3 target antigens for T cells in normal mouse ovaries. In that oophoritis develops in mice immunized with ZP3$^{328-342}$, we surmise that endogenous ZP3 protein must be processed by antigen-presenting cells in the normal mouse ovaries. The antigen-presenting cells then present the peptide to the peptide-specific CD4$^+$ T cell in vivo. Normal mouse ovaries contain abundant macrophages that constitutively express readily detectable class II MHC molecules (21). They exist in interfollicular areas and inside atretic ovarian follicles, the frequent sites of tissue injury in autoimmune oophoritis. In contrast, class II MHC molecules have not been detected on oocytes in normal ovary (22) or in ovaries with murine autoimmune oophoritis (K. S. K. Tung, unpublished data).

Autoimmune disease involving the human ovary can occur in conjunction with autoimmune disease in other endocrine organs or as an isolated entity (1–3, 23). In either event, the oophoritis is characterized by lymphocytic infiltration of the ovary that preferentially destroys mature follicles. As the disease progresses, the ovary becomes atrophic and this is associated clinically with premature ovarian failure and sterility. Ovarian autoantibodies are detected in the circulation of affected patients (24) and the relative contribution of antibody and T cell response to the ovarian disease remains obscure. Although human autoimmune oophoritis most likely results from diverse etiologies, the identification of an oophoritogenic epitope in the zona pellucida of mice may lead to a better understanding of how autoimmune T cells mediate human ovarian autoimmune disease. Such investigations will be facilitated by the recent isolation of the human ZP3 gene and the definition of primary structure of the ZP3 protein (17). Whether T cell response to the ZP3 peptide is important in the pathogenesis of oophoritis in neonatally thymectomized mice has not yet been fully investigated.

These studies have implications beyond the understanding of the pathogenesis of autoimmune oophoritis. Investigations designed to test the potential of zona pellucida as a target for immunocontraception have been complicated by the loss of ovarian function and development of ovarian histopathology

after immunization with zona proteins (25). Insofar as we have shown that immunization with a ZP3 peptide can result in oophoritis in mice, it is probable that the ovarian disease reported in other species represents the end stage of autoimmune oophoritis. Based on this and an earlier study (20), we suggest that antibody to ZP3 may cause reversible infertility while T cell response to ZP3 is responsible for oophoritis. The precise definition of an oophoritogenic peptide in a region of ZP3 known to contain binding sites for antibodies against the zona pellucida should facilitate the rational design of contraceptive vaccines that will selectively induce antibodies but not cause T cell–mediated ovarian damage. Although the genetic variation in antibody response poses a potential problem for a ZP3 peptide vaccine, this may be overcome by conjugation of the peptide to an immunogenic carrier protein.

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