Antigen Mimicry in Autoimmune Disease
Sharing of Amino Acid Residues Critical for Pathogenic T Cell Activation

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Abstract

A nonamer peptide from murine nicotinic acetylcholine receptor δ chain (ACRδ), which shared four amino acid residues with a nonamer peptide of murine ovarian zona pellucida glycoprotein ZP3, induced murine autoimmune oophoritis and IgG autoantibody to the zona pellucida. Crossreaction between the ACRδ and ZP3 peptides was established by the response of a ZP3 peptide–specific, oophoritogenic T cell clone to both peptides in association with IA($\alpha^k\beta^b$). By substituting the ZP3 peptides with a single alanine, four amino acids within the ZP3 peptide were found to be important for ovarian autoimmune disease, autoantibody response, and stimulation of the ZP3-specific T cell clone. Substitution with conservative amino acids of three residues also ablated activity, whereas the fourth, a phenylalanine, was replaceable by tyrosine without loss of activity. Of the four critical amino acids, three were shared between the ZP3 peptide and the ACRδ peptide. Moreover, polyalanine peptides with the four critical ZP3 amino acids or the four amino acids common to the ZP3 and ACRδ peptides induced immune response to ZP3 and elicited severe ovarian autoimmune disease. Thus, organ-specific autoimmune disease can occur through immune response against unrelated self (or foreign) peptides that share with a self-peptide sufficient common amino acid residues critical for activation of pathogenic, autoreactive T cells. (*J. Clin. Invest.* 1993. 92:2117–2123.) Key words: autoimmune disease • premature ovarian failure • myasthenia gravis • molecular mimicry • peptide vaccines

Introduction

Antigen mimicry has long been proposed as a potential mechanism underlying organ-specific human autoimmune diseases (1). Idiopathic encephalitis, neuritis, and myelitis often occur after viral infection, and autoantibodies have been detected in these patients that react with neural antigens. Investigations in the past have focused on foreign antigenic determinants recognized by autoantibodies (2). Although foreign antigens induced antibody responses that crossreacted with self molecules, autoimmune disease rarely developed in the immunized animals. There are some exceptions. For example, focal encephalomyelitis was induced in rabbits immunized with a peptide from hepatitis B virus; the viral peptide shared common sequence with, and provoked antibody against, the myelin basic protein (3). Another was the induction of myasthenia gravis in rabbits immunized with a chemical analogue of acetylcholine, through induction of antiidiotype antibody that crossreacted with the nicotinic acetylcholine receptor (ACR)[1] (4).

Since activation of T cell response to self-peptides is a pivotal event in the pathogenesis of organ-specific autoimmune diseases, crossreaction between foreign and self-peptides recognized by T cells should, in principle, be more pertinent than autoantibodies in antigen mimicry (5). We have therefore explored this possibility based on a model of murine ovarian autoimmune disease (oophoritis) elicited by a well-characterized ovarian peptide (6).

ZP3 is a glycoprotein in the zona pellucida, its O-linked oligosaccharide being the major sperm receptor in fertilization (7). Within the murine ZP3 sequence (8) is a 13-mer ZP3 peptide (ZP3 330–342) that has two overlapping antigenic domains: ZP3 336–342, a 7-mer linear sequence recognized by ZP3 antibody (9), and ZP3 330–337, the minimal octomer sequence that induces oophoritis, pathology transferrable by pathogenic CD4$^+$ T cells (6). Murine autoimmune oophoritis is a model of human premature ovarian failure in which oophoritis and autoantibodies to ovarian antigens have been documented (10). In addition, premature ovarian failure often coexists with autoimmune adrenalitis, autoimmune thyroiditis, and myasthenia gravis (11). It is currently unclear as to why multiple autoimmune diseases should occur in the same individual. In this paper, we present evidence for antigen mimicry between the murine ZP3 peptide and a peptide from the murine ACRδ chain (ACRδ). Mice immunized with the nonamer ACRδ peptide developed oophoritis and autoantibodies to the zona pellucida. The evidence for, and the mechanism underlying, the crossreaction between the ZP3 and the ACRδ peptides will be presented.

Methods

Induction of murine autoimmune oophoritis. Adult female (C57BL/6 × A/J)F_1 ($B6AF_1$) mice were purchased from The Jackson Laboratory (Bar Harbor, ME) or the National Cancer Institute (Frederick, MD). Each peptide (at 50-μg dose, dissolved in deionized/distilled water) was emulsified in an equal volume of CFA containing 1 mg/ml of *Mycobacterium tuberculosis* (H37Ra; Difco Laboratories, Detroit, MI) or in incomplete Freund's adjuvant (ICFA). Each mouse received 0.1 ml of the peptide and adjuvant emulsion, distributed in the two hind footpads. Histologic evidence of autoimmune oophoritis was determined 14 d later.

Histology and immunohistochemistry. The ovaries fixed in the Bouin's fixative were embedded in paraffin. Approximately 50 serial step sections per ovary, 5 μm thick, were stained with hematoxylin and

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1. *Abbreviations used in this paper:* ACR, ACRδ, and ACRγ, acetylcholine receptor, δ and γ chains; EAE, experimental autoimmune encephalomyelitis.

eosin. Histopathology of oophoritis was evaluated as coded specimens, with severity graded 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 (6).

For direct immunofluorescence study, frozen mouse ovaries were embedded in OCT compound, and 5-μm-thick sections were cut in a cryostat. After the tissue sections were fixed in 90% ethanol for 15 min, they were rinsed in PBS and then incubated with fluorescein isothiocyanate–conjugated goat antiserum IgG containing antibody to mouse Igs, IgG heavy chain or IgM heavy chain (Southern Biotechnology, Birmingham, AL) for 30 min. After the sections were rinsed in PBS, a coverslip was applied over glycerol containing 10% PBS; the images were examined and photographed with a fluorescence microscope (Olympus Corp. of America, New Hyde Park, NY).

Indirect immunoperoxidase technique was used to detect infiltrating T cells in frozen ovarian sections with the hamster monoclonal anti-mouse CD3 antibody (145-2C11), followed by biotinylated goat antiserum IgG to hamster IgG (Vector Laboratories, Inc., Burlingame, CA). Peroxidase/avidin complex (Vector Laboratories, Inc.) was then applied, and the tissue-bound enzyme was visualized after the tissue section was incubated with 3,3′-diaminobenzidine and fresh H_2O_2 (Sigma Chemical Co., St. Louis, MO); the substrate color was intensified by exposure to osmium vapor.

Peptide synthesis, purification, and characterization. Peptides were synthesized by the solid-phase method using the RAMPS system (DuPont Corp., Boston, MA) and purified by high pressure liquid chromatography on a C18 reverse phase column (Waters Associates, Milford, MA). The purity of all the peptides exceeded 90%. The amino acid sequence was confirmed by mass spectroscopy (12).

Production and proliferation assay of a ZP3 330–342–specific T cell clone. The ZP3 330–342–specific T cell line was produced as described previously (6). Briefly, lymph node cells from B6AF$_1$ mice immunized with 100 μg of ZP3 328–342 in CFA were stimulated in vitro with 30 μM of ZP3 330–342 in RPMI 1640 supplemented with 1% sodium pyruvate, 1% nonessential amino acids, 2 mM glutamine, 100 U penicillin, 100 μg/ml streptomycin, and 5×10^{-5} M 2-mercaptoethanol (Gibco Laboratories, Grand Island, MI), with 10% heat-inactivated FCS (Sigma Chemical Co.) (complete medium). 4 d later, the lymphocytes were rested in complete medium with 1–2% of lymphokine-enriched supernatant of the cultured EL4 thymoma cell line containing 1.5 U of IL-2 activity. After 10 d, viable lymphocytes were restimulated with 30 μM of ZP3 330–342 in complete medium, and rested as above. After four cycles of alternating cell stimulation and rest, a cell line was established that responded specifically to ZP3 330–342. From the T cell line, a ZP3 330–342–specific T cell clone (J3) was obtained by limiting dilution at 0.3 cells per each 200-μl flat-bottomed well in complete medium containing 1–2% of EL4 cell culture supernatant. When the J3 clone reached the growth phase, it was recloned as above at 0.3 cells per well.

To measure the proliferative response of the J3 clone, 5×10^4 J3 cells were cultured with 5×10^5 antigen-presenting cells and the stimulating peptide at several peptide concentrations in 200 μl of complete medium. After 4 d in 37°C in 5% CO_2 and air, 0.5 μCi of [^{3}H]-thymidine was added; and cell-associated radioactivity was determined in a β counter 8 h later. Data were expressed as delta counts per minute (Δcpm) (cpm in T cells cultured with antigenic peptide − cpm in T cells cultured without antigenic peptide).

The following antigen-presenting cells were used to determine the MHC restriction of the J3 clone in its response to the ZP3 peptide: irradiated spleen cells from B6AF$_1$, A/J, C57BL/6, or B10A.5R mice, mitomycin-treated L cells transfected with murine IA($\alpha^k\beta^b$) or with murine IA($\alpha^b\beta^k$) (a generous gift of Dr. Ned Braunstein, Columbia University, New York, NY).

Adoptive transfer of autoimmune oophoritis by the J3 clone. After the ZP3-specific T cell clone (J3) was stimulated in vitro with 30 μM of ZP3 330–342 for 4 d, the viable T cell blasts isolated in a Hypaque-Ficoll gradient were injected intraperitoneally into normal, untreated adult B6AF$_1$ female mice. Ovarian pathology in cell recipients was examined 10 d after cell transfer.

Cytokine assays. IL-2, IL-4, tumor necrosis factor, and interferon γ were quantitated as described elsewhere (13).

Statistical analysis. Significance in disease incidence differences was determined by the chi-square analysis.

Results

A peptide from murine ACR delta chain elicits murine autoimmune oophoritis. Among truncated ZP3 328–342 peptides, ZP3 330–338 was the shortest oophoritogenic peptide found to stimulate mitogenic response of an oophoritogenic T cell line (6). A search in the protein sequence library revealed two murine ACR peptides that share partial sequence homology with ZP3 330–338: ACRδ 120–128 and an ACRγ peptide, ACRγ 117–124 (Table I) (14, 15). This finding was of potential interest because of the known clinical association between premature ovarian failure and myasthenia gravis.

The peptides ACRδ 120–128 and ZP3 330–338 share four common amino acid residues. When B6AF$_1$ mice were immunized with ACRδ 120–128, they developed oophoritis (Table I). In contrast, B6AF$_1$ mice immunized with ACRγ 117–124, which shared only two common amino acids with ZP3 330–338, did not develop ovarian pathology.

In mice immunized with ACRδ 120–128, inflammatory infiltrates were noted in ovarian interfollicular spaces and within the Graafian follicles. The infiltrates contained macrophages, multinucleated giant cells, and lymphocytes (Fig. 1 B), of which many were T (CD3$^+$) cells (Fig. 1 C). The nature and the extent of ovarian histopathology in the ACRδ 120–128–immunized mice resembled the mild to moderate oophoritis observed in mice immunized with ZP3 330–338 (6). Consistent with oophoritis induced by the ZP3 peptides, mice immunized with ACRδ 120–128 in ICFA also developed ovarian autoimmune disease (6) (Table I).

Although ZP3 330–338 stimulated the ZP3 330–342–specific T cell line to proliferate, T cells from regional lymph nodes of mice immunized with ZP3 330–338 did not proliferate against ZP3 330–338 in vitro (6). We therefore generated an oophoritogenic, ZP3 330–342–specific T cell clone for purpose of exploring T cell receptor recognition of the ZP3 peptide and the ACRδ peptide.

Production and characterization of a murine ZP3 peptide–specific oophoritogenic T cell clone. From an oophoritogenic ZP3-specific T cell line (6), a ZP3 330–342–specific T cell clone (J3) was obtained by limiting dilution, twice, at 0.3 cells per well. The J3 clone was CD4$^+$; upon activation, it produced IL-2, interferon γ, and tumor necrosis factor, but not IL-4 (data not shown). As shown in Table II, the J3 clone adoptively transferred oophoritis to untreated B6AF$_1$ adult recipients, but did not transfer orchitis to B6AF$_1$ male recipients. We next determined MHC restriction of ZP3 peptide recognition by this clone.

The functional class II MHC molecules expressed on a B6AF$_1$ antigen-presenting cell include IA($\alpha^k\beta^k$), IA($\alpha^b\beta^b$), IA($\alpha^k\beta^b$), IA($\alpha^b\beta^k$), IE($\alpha^k\beta^k$), and IE($\alpha^k\beta^b$). The J3 clone was found to respond to the ZP3 330–342 only in the presence of irradiated B6AF$_1$ spleen cells (mean Δcpm, 138,640) or mitomycin-treated L cells transfected with IA($\alpha^k\beta^b$) (mean Δcpm, 93,692). No response to ZP3 330–342 occurred in the

Table I. Crossreaction between Murine ZP3 Peptide and Murine Acetylcholine Receptor Delta Chain Peptide for Induction of Autoimmune Oophoritis and Ovary-bound Anti-Zona Pellucida Antibody Response, and for Stimulation of an Oophoritogenic T Cell Clone

Adjuvant	Peptide	Sequence	Oophoritis Incidence (%)	Oophoritis Severity	ZP-bound IgG	Proliferative responses of the J3 clone
				1 2 3 4		*Δcpm*
CFA	None		0/5		0/5	302
CFA	ZP3 330–338	NSSSSQFQI	5/5 (100)	0 2 3 0	5/5	121,222
CFA	mACRδ 120–128	NNNDGSFQI	3/5 (60)	1 2 0 0	3/5	95,659
CFA	mACRγ 117–124	NNVDGVFEV	0/5		0/5	−2,097
CFA	None		0/5		0/5	243
CFA	ZP3 330–338	NSSSSQFQI	4/5 (80)	1 1 2 0	5/5	51,364
CFA	mACRδ 120–128	NNNDGSFQI	2/5 (40)	1 1 0 0	3/5	15,397
CFA	mACRγ 117–124	NNVDGVFEV	0/5		0/5	−2,734
ICFA	None		0/5		0/5	276
ICFA	ZP3 330–338	NSSSSQFQI	4/4 (100)	1 1 2 0	4/4	249,314
ICFA	mACRδ 120–128	NNNDGSFQI	3/4 (75)	1 1 1 0	3/4	22,021
ICFA	mACRγ 117–124	NNVDGVFEV	0/5		0/5	−2,683

In three independent experiments, adult female $B6AF_1$ mice immunized with 50 μg of the peptide in adjuvant, or adjuvant alone, were studied 14 d later. Antibody to zona pellucida is detected by direct immunofluorescence as intense IgG bound to ovarian zona pellucida. The proliferative responses of the ZP3 330–342–specific, oophoritogenic T cell clone, J3, to the peptides (30 μM) in presence of mitomycin-treated L cells transfected with IA($\alpha^k\beta^b$) are expressed as Δcpm.

presence of antigen-presenting cells from A/J (mean Δcpm, 153), C57BL/6 (mean Δcpm, 94), or B10.A(5R) (mean Δcpm, 93) mice, or with L cells transfected with IA($\alpha^b\beta^k$) (mean Δcpm, −152). The recognition of the ZP3 330–342 by the J3 clone was therefore restricted to IA($\alpha^k\beta^b$). In addition, it was found that 100-fold less ZP3 330–338 was required to stimulate J3 in the presence of the L cells transfected with IA($\alpha^k\beta^b$) than in the presence of $B6AF_1$ spleen cells (data not shown). We therefore used the transfected L cells as antigen-presenting cells in subsequent studies.

Z P3 330–342–specific oophoritogenic T cell clone responds to ACRδ 120–128. The oophoritogenic J3 T cell clone proliferated in response to ZP3 330–342 and ACRδ 120–128, but not to ACRγ 117–124 (Table I). Although J3 responded more vigorously against ZP3 330–338 than against ACRγ 120–128, the finding establishes crossreaction in T cell recognition between ZP3 330–338 and ACRδ 120–128.

Mice immunized with ZP3 330–338 or ACRδ 120–128 produce autoantibody to the zona pellucida. Antibody to the zona pellucida, of IgG and not IgM class, was detected in ovaries of mice immunized with ACRδ 120–128 or ZP3 330–338, but not in mice immunized with ACRγ 117–124 (Table I). The antibody, detectable by direct immunofluorescence, appeared as intense IgG staining in the ovarian zona pellucida (Fig. 1 *D*).

Because ZP3 330–338 is known to have T cell but not B cell epitope (15a), induction of antibody to the zona pellucida by ZP3 330–338 or ACRδ 120–128 was unexpected. Although we will defer a discussion on the basis for the unexpected autoantibody response to the Discussion section, the induction of T cell–dependent IgG antibody response to zona pellucida by ACRδ 120–128 provides evidence for the crossreaction between ACRδ 120–128 and ZP3 330–338 in stimulating helper T cell required for anti–zona pellucida IgG antibody response. Quantitative analysis of the IgG eluted from the ovaries also established that the zona-bound IgG in mice immunized with the ZP3 peptide was highly enriched (~ 200–400) for antibody to the zona pellucida over serum IgG (15a).

Studies of ZP3 330–338 peptides with a single alanine substitution. We next identified the amino acid residues in ZP3 330–338 important for induction of (*a*) autoimmune oophoritis, (*b*) anti–zona pellucida antibody, and (*c*) mitogenic response of the oophoritogenic T cell clone.

The ZP3 330–338 peptide in which the residue Asn 330, Gln 335, or Phe 336 was substituted by an alanine did not elicit autoimmune oophoritis (Table III, Study 1). The replacement of Gln 337 by alanine also reduced the oophoritogenic potential of ZP3 330–338 significantly ($P = 0.01$). Mice immunized with these four alanine-substituted ZP3 peptides likewise did not develop antibody to the zona pellucida (Table III). However, when the other amino acid residues of ZP3 330–338 were each substituted by an alanine, the peptides retained their capacity to induce autoimmune oophoritis and zona pellucida antibody (P values ranged from 0.4 to 0.8) (Table III, Study 1).

The proliferative responses of the oophoritogenic T cell clone to single alanine–substituted ZP3 330–338 were then examined. J3 clone did not respond to ZP3 330–338 with the Phe 336-to-Ala substitution, even at a 100-μM peptide concentration. The responses to ZP3 330–338 with alanine substitution at residue Asn 330, Gln 335, or Gln 337 were significantly reduced; the J3 clone proliferated only at 100-μM concentration. In contrast, the responses of the J3 clone to ZP3 330–338 with alanine substitution in position Ser 331, Ser 332, Ser 333, Ser 334, or Ile 338 were similar to the response to ZP3 330–338 occurring at 1-μM peptide concentration (Table III, Study 1). Thus, the four amino acid residues common to ZP3 330–338 and ACRδ 120–128 included three residues (Asn 330, Phe 336, Gln 337) that were required for activating the oophoritogenic, ZP3 peptide–specific T cell clone.

Studies of ZP3 330–338 substituted with conservative amino acids. We next studied ZP3 330–338 with the following

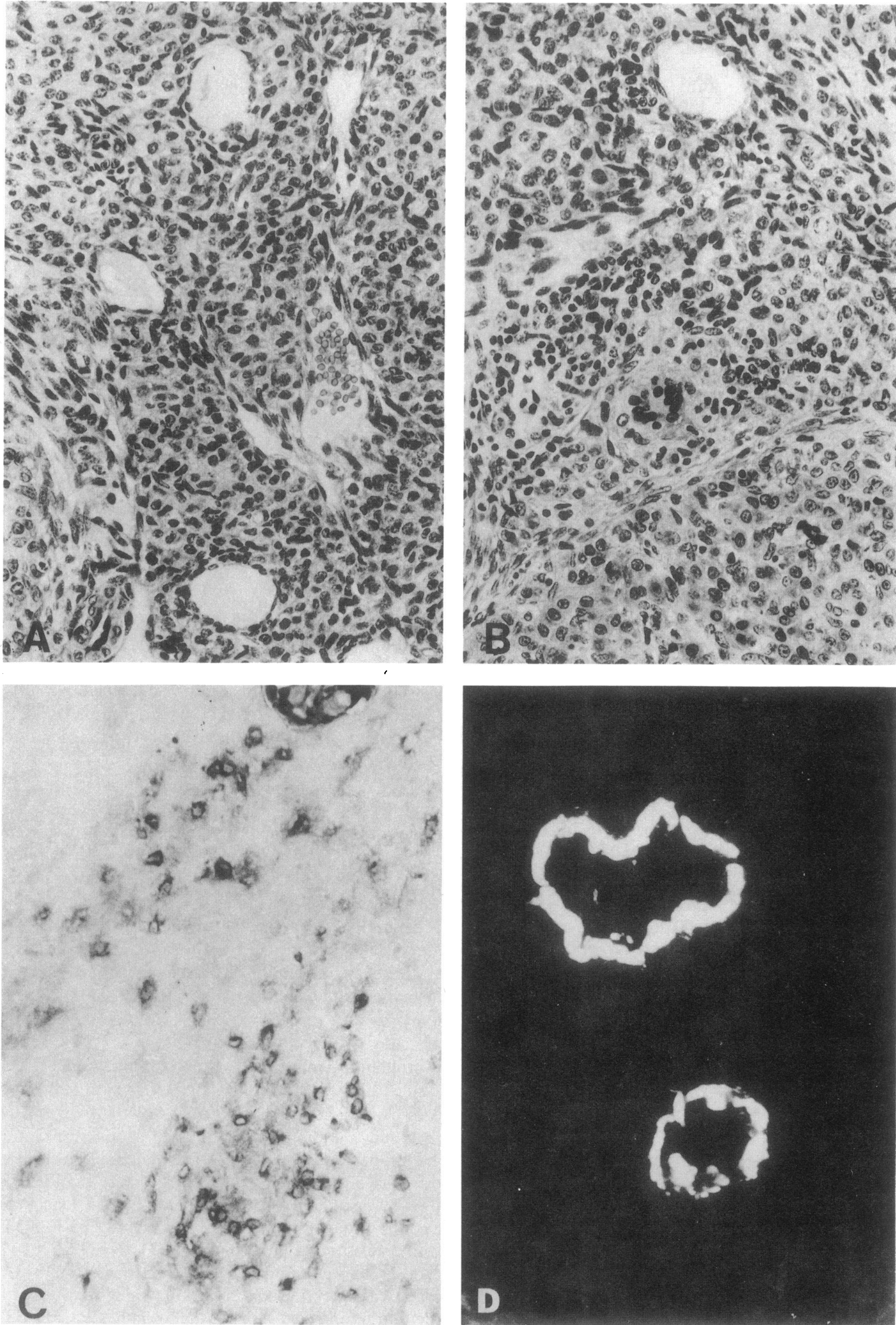

Figure 1. Ovarian immunopathology of a mouse immunized with the murine nicotinic acetylcholine receptor peptide, ACRδ 120–128. Compared with normal mouse ovary (*A*), which is free of identifiable inflammatory leukocytes, the ovarian interstitial space of a mouse immunized with ACRδ 120–128 in CFA contains infiltrates of mononuclear inflammatory cells that include a multinucleated giant cell (*B*). In a mouse immunized with the ACRδ 120–128 in CFA, T ($CD3^+$) cells identified by immunoperoxidase are found in the ovarian inflammatory infiltrate (*C*), and intense staining of mouse IgG (zona pellucida antibody) is detectable by direct immunofluorescence in the ovarian zona pellucida (*D*) (*A* and *B*, ×100; *C* and *D*, ×400).

Table II. Adoptive Transfer of Autoimmune Oophoritis by the ZP3 330–342 Peptide-specific T Cell Clone to Normal B6AF₁ Mice

T cell clone	Antigen specificity	Cell number	Pathology incidence	
			Oophoritis	Orchitis
		$\times 10^6$		
J3	ZP3 330–342	1–20	26/29 (90%)	0/10
D6	Testis homogenate	10	0/6	29/38 (76%)

J3 clone was obtained from $B6AF_1$ female mice and D6 clone from male $B6AF_1$ mice. Generation and characterization of the D6 clone have been described (13). T cell clones were injected intraperitoneally and histopathology of the testis and the ovary was evaluated 10 d later. Recipients of both clones were free of pathology outside the gonads.

conservative single amino acid substitutions: Asn 330 with Gln, Gln 335 with Asn, Phe 336 with Tyr, Gln 337 with Asn. Peptides with substitution at Asn 330, Gln 335, or Gln 337 did not elicit autoimmune oophoritis or autoantibody to the zona pellucida; and they did not stimulate the J3 clone (Table III, Study 2). In contrast, the peptide with the Phe 336-to-Tyr substitution retained full capacity to induce oophoritis and autoantibody response to zona pellucida, and it stimulated proliferation of the J3 clone at peptide concentration of 0.1 μM (Table III, Study 2).

Study of polyalanine peptides with insertion of selected amino acid residues of ZP3 330–338 and of ACRδ 120–128. Here, we positively identify the amino acid residues in ZP3 330–338 and ACRδ 120–128 that are sufficient to elicit autoimmune oophoritis, anti-zona pellucida antibody response, and stimulation of the oophoritogenic J3 clone.

We first studied the substituted polyalanine peptide, NAAAAQFQA, which contained the four amino acid residues in ZP3 330–338 shown by the single alanine scan to be important for inducing autoimmune oophoritis. $B6AF_1$ mice immunized with the NAAAAQFQA peptide developed severe oophoritis and autoantibody to zona pellucida (Table III, Study 3). We next studied the polyalanine peptide that contained the amino acids common to ZP3 330–338 and ACRδ 120–128 (NAAAAAFQI). The NAAAAAFQI peptide also induced severe oophoritis and autoantibody response to the zona pellucida, and the ovarian pathology was actually more severe than those observed in mice immunized with ACRδ 120–128. In parallel, the J3 clone responded vigorously to both NAAAAQFQA and NAAAAAFQI peptides (Table III, Study 3). In contrast, the peptide NAAAAAFAA, which contained the two common amino acid residues shared between ZP3 330–338 and ACRγ 117–124, did not induce ovarian disease or autoantibody, nor did not stimulate the J3 clone to proliferate.

Discussion

This study has demonstrated that a nonamer peptide from mouse ACRδ, which has four amino acid residues in common with a mouse ovarian ZP3 nonamer peptide, is recognized by a pathogenic ZP3 peptide-specific T cell clone, and the recognition was restricted by the same class II MHC molecule. Importantly, $B6AF_1$ mice actively immunized with the ACRδ peptide developed ovarian autoimmune disease. Their ovarian inflammatory infiltrates contained numerous T cells, and they developed autoantibody of the IgG class that bound to the zona

Table III. Mapping the Amino Acid Residues in ZP3 330–338 Important for Induction of Autoimmune Oophoritis and Anti-Zona Pellucida Antibodies, and for Stimulation of an Oophoritogenic T Cell Clone

Study	Peptide sequence	Oophoritis		Zona-bound IgG	Proliferation of J3 clone (Δcpm)			
		Incidence	Severity		0.1	1	10	100
		%	1 2 3 4		Peptide, μM			
1	NSSSSQFQI	9/10 (90)	1 3 5 0	10/10	169	12,216	32,874	249,315
	ASSSSQFQI	0/9 (0)	0 0 0 0	0/9	−185	518	600	46,739
	NASSSQFQI	8/9 (90)	2 3 2 1	6/9	−56	25,924	51,738	236,450
	NSASSQFQI	10/10 (100)	2 2 6 0	4/5	1,756	34,433	85,815	219,133
	NSSASQFQI	5/5 (100)	0 1 3 1	5/5	835	44,120	79,919	167,197
	NSSSAQFQI	6/10 (60)	2 2 2 0	3/5	−167	1,046	34,234	141,256
	NSSSSAFQI	0/9 (0)	0 0 0 0	0/5	−194	−206	637	15,810
	NSSSSQAQI	0/9 (0)	0 0 0 0	0/9	−218	−225	86	398
	NSSSSQFAI	4/10 (40)	3 1 0 0	2/5	−211	−225	−33	7,048
	NSSSSQFQA	5/5 (100)	0 1 2 2	4/5	36,211	37,337	211,861	241,213
2	QSSSSQFQI	0/5 (0)		0/5	−163	3	259	997
	NSSSSNFQI	0/5 (0)		0/5	−3	348	964	2,142
	NSSSSQYQI	3/5 (60)	0 1 1 1	4/5	6,262	28,758	197,601	310,881
	NSSSSQFNI	0/5 (0)		0/5	25	1,663	1,663	3,082
3	NAAAAQFQA	8/10 (80)	3 3 2 0	8/10	27,284	109,783	158,031	89,808
	NAAAAAFQI	13/15 (87)	3 7 3 0	15/15	30,769	64,960	124,713	121,605
	NAAAAAFAA	0/10 (0)		0/7	−2,615	−2,039	1,295	11,456

The results of the in vivo experiments in studies 1 and 3 are pooled from two independent experiments; those of study 2 are from one experiment. The proliferative responses of the J3 clone to the various peptides are highly reproducible and the result of a representative experiment is shown.

pellucida in vivo. Of the four amino acids shared by the two unrelated self peptides, three were important for (*a*) activation of the oophoritogenic T cell clone, (*b*) induction of autoimmune oophoritis, and (*c*) development of zona pellucida autoantibodies. To address the evidence for T cell mimicry directly, we studied a polyalanine peptide with the shared amino acid residues between the ACRδ and the ZP3 peptides. We then compared this peptide with the polyalanine peptide that contained the four critical amino acid residues of the ZP3 peptide. Both substituted polyalanine peptides were as active as the nonamer ZP3 peptide with respect to oophoritis and autoantibody induction, and in stimulation of the oophoritogenic T cell clone. It would appear that the sharing of the three critical amino acid residues between the ZP3 and ACRδ peptides might be the minimal requirement for oophoritis induction. The murine ACRγ peptide, which had only two of the critical amino acid residues in common with the ZP3 peptide, did not induce disease or autoimmune responses to ZP3. We conclude that the ACRδ peptide elicits ovarian autoimmune disease through activation of ZP3 330–338-specific pathogenic T cells, and this occurs when the two unrelated peptides share sufficient critical amino acid residues to stimulate a pathogenic T cell. We should emphasize that pathogenic self-reactive T cells against ovarian antigen exist in normal mice since mature $CD4^+$ thymocytes and T cells in the peripheral T cell pool of normal adult female mice, when transferred to athymic recipients, caused autoimmune oophoritis (16).

The induction of antibody response to the zona pellucida by ACRδ 120–128 was an unexpected but an important observation. We have recently shown that mice immunized with oophoritogenic ZP3 peptides that contained T cell epitope but not B cell epitope can elicit antibody response to antigenic determinants of the ZP3 protein outside the immunizing peptide (15a). The study provides evidence that in the presence of endogenous ovarian antigens, activation of ZP3-specific T cell per se is sufficient to provide the helper T cell signal for an autoantibody response against zona pellucida antigens, including ZP3. First, the truncated ZP3 T cell peptides that lack the region 336–342 were found not to have additional B cell epitopes; indeed, the region 336–342 was the only B cell epitope in ZP3 330–342 recognized in native zona pellucida. Second, antibodies to the zona pellucida were demonstrated both in the serum and ovarian zona pellucida of mice immunized with these truncated ZP3 peptides; and by immunoblot analysis, the serum antibody reacted with ZP3. Third, IgG eluted from the zona pellucida was enriched 200–400-fold in antibody activity to the zona pellucida over serum IgG, as determined by immunofluorescence and ELISA, but was not enriched for antibody to the 336–342 determinant. Finally, when ovariectomized mice were immunized with the ZP3 T cell peptide, they did not have serum antibody to the zona pellucida, thus the endogenous ovarian zona pellucida is the likely source of antigenic stimulus for the amplified autoantibody response to the zona pellucida.

The finding that ACRδ 120–128 also has the capacity to induce autoantibody to the zona pellucida provides strong evidence that the ACRδ peptide is also recognized by, and can activate, ZP3 peptide-specific helper T cells. Even more important, it demonstrates clearly that mimicry strictly at the level of T cell epitope can lead to immunologic events beyond the induction of T cell response and autoimmune disease. That is, T cell epitope mimicry alone can evoke an autoantibody response against the native protein antigen. Therefore, it is quite possible that research that focuses on autoantibodies could minimize or mask the importance of T cell–mediated immunity in organ-specific, and perhaps systemic, autoimmune disease. On the other hand, the zona pellucida autoantibodies in mice immunized with a T cell peptide of ZP3 preferentially reacted with the native determinants of ZP3 and were bound to the zona pellucida in vivo; thus they are potentially relevant in autoimmune disease pathogenesis (15a). We have recently uncovered antigen mimicry between ZP3 330–338 and exogenous antigenic peptides (Garza and Tung, preliminary findings). They include (*a*) a torpedo ACRγ peptide, which shared three of the four critical amino acids with ZP3 330–338 but differs from murine ACRγ 117–124 in one of these three amino acid residues, and (*b*) a viral peptide. Immunization of $B6AF_1$ mice with each of these exogenous peptides led to autoimmune oophoritis and autoantibodies to the zona pellucida. Thus, the mechanism of antigen mimicry between ZP3 330–338 and ACRδ 120–128 does not appear to be an isolated experimental observation.

We have not yet determined whether the critical amino acid residues in the ZP3 330–338 peptide are required for binding to IA($\alpha^k\beta^b$) or as contact sites with the oophoritogenic T cell receptor. In this regard, the finding on the phenylalanine residue in ZP3 336 is of interest. The oophoritogenicity of ZP3 330–338 was lost when Phe 336 was replaced by alanine but not by tyrosine. We have since obtained evidence that the ZP3 peptide retained oophoritogenic activity when Phe 336 was replaced with hydrophobic but not polar amino acid residues (Garza and Tung, preliminary findings). Thus, Phe 336 may be critical by virtue of the chemical property required for interaction with the MHC molecule or the T cell receptor. The finding of amino acid residues in T cell epitopes with functions replaceable by some but not other unrelated amino acids is not new (17), and has also been documented in another self peptide of the myelin basic protein (18).

Antigen mimicry has long been proposed as an event that might trigger human organ-specific autoimmune diseases (19). The possibility that autoimmune disease could be induced through antigen mimicry of T cell epitopes was initially documented in study on a peptide from a *Escherichia coli* protein. The peptide was found to share sequence homology with the S retinal protein, and rats immunized with the *E. coli* peptide led to autoimmune uveitis and T cell response to the S peptide (20). However, the study neither established T cell cross-reaction between the peptides nor investigated the mechanism behind the observed phenomenon. Moreover, very large doses of the *E. coli* peptide (400–2,000 μg) were required to elicit eye pathology. Two recent studies have also explored T cell epitope mimicry by a novel approach. On the basis of the protocol that recreated the T cell epitopy of a ribonuclease peptide (21), polyalanine peptides were synthesized that contained the critical amino acid residues of the encephalitogenic peptide of the myelin basic protein (18, 22). These peptides not only stimulated proliferation of encephalitogenic T cell clone, they induced experimental autoimmune encephalomyelitis (EAE) as well.

The impetus behind our study was initially to elucidate the mechanism for concurrence of autoimmune ovarian disease and myasthenia gravis. Whereas antigen mimicry remains a potential mechanism behind multiple clinical autoimmune diseases, the recently published amino acid sequence of human ZP3 has ruled out direct extrapolation of our experimental findings to the concurrence of the two human diseases (23).

The murine and human ZP3 330–338 sequences are highly variant, whereas their ACRδ 120–128 sequences are identical.

This study has important implications for peptide vaccines from foreign proteins that elicit T cell responses, where, similar to experimental autoimmune disease induction, individuals would be vaccinated with a peptide (24). On the basis of this and other studies (18, 22), it is clear that molecular mimicry for autoimmune disease induction requires merely a few randomly positioned amino acid residues (< 50%) of a self-peptide. Therefore, the potential target self-peptides are not likely detectable by the vaccine peptide probes (antibody or oligonucleotide). Moreover, even if the critical amino acid residues in the vaccine peptide for T cell activation are known, to search for self target peptides with shared amino acid residues is experimentally difficult if not impossible. Finally, powerful adjuvants such as myobacterial components or pertussis toxin are not always required for induction of experimental autoimmune diseases. Although they are obligatory for induction of murine EAE (19) and murine experimental autoimmune orchitis (25), they are not required for murine autoimmune oophoritis (6).

With respect to antigen mimicry in spontaneous autoimmune diseases, it may be argued that immune response to a small peptide does not represent a likely event by which the human disease is triggered. In responses against self proteins that are subjected to tolerance regulation, there is evidence that the pathogenic peptides within a self-protein may be cryptic. Their immunogenicity is manifested only when presented to the host as synthetic peptides and not as the natural sequences of the whole protein (26, 27). However, this finding may not apply to responses against T cell epitopes in microbial protein antigens. Spontaneous immune response to microbial agents is complex; and infections, through unknown mechanisms, have been found to terminate an experimentally induced tolerance state (28–30). Microbial superantigens may bypass the tolerance state by coactivating Vβ-specific self-reactive T cells (31). Therefore, whether a peptide in a microbe that mimics a self peptide can function as a potent immunogen during an infection remains to be determined experimentally.

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