

FINE SPECIFICITY OF GENETIC REGULATION OF
GUINEA PIG T LYMPHOCYTE RESPONSES
TO ANGIOTENSIN II AND RELATED PEPTIDES*

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One approach to investigating the nature of T lymphocyte recognition of exogenous antigens and mechanisms of Ir gene control has used small, well-defined peptide antigens. In previous studies we used synthetic homologues and analogues of human fibrinopeptide B to examine strain 2 and strain 13 guinea pig T cell responses and found that Ir gene control correlated with the presence or absence of the carboxyl terminal residue, and that responsiveness was determined by macrophage Ia antigens (1, 2). In addition, several peptide residues were identified that were responsible for the specificity of the T cell responses, and most likely served as contact residues for clonally distributed T cell antigen-combining receptors (3). However, these immune responses were solely cell mediated, and we were unable to generate detectable antibody by a variety of approaches. It was therefore difficult to compare T and B cell recognition of the same peptide antigen to determine whether the antigen-combining repertoire of both cell types was similar. For this reason, we have employed the octapeptide hormone angiotensin II (AII)¹ as an antigen system to investigate T and B cell recognition. AII has been used previously to investigate the specificity and spatial constraints of antibody binding in several species (reviewed in reference 4). Moreover, Dietrich (5) found that free AII elicited both immediate and delayed-type hypersensitivity reactions in guinea pigs. In this study we have extended the findings of Dietrich to examine Ir gene control and the specificity of T cell responses to a variety of synthetic homologues and analogues of AII in strain 2 and strain 13 guinea pigs. Evidence is presented that demonstrates the exquisite specificity of Ir gene control of T cell responses and indicates that the diversity of the antigen recognition repertoire in strain 2 and strain 13 animals is generally nonoverlapping.

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¹ *Abbreviations used in this paper:* Ahp, heptyline, L-2-aminoheptanoic acid; AI, angiotensin I; AII, angiotensin II; AIII, [des-Asp¹]-AII; CFA, complete Freund's adjuvant; [³H]TdR, [methyl-³H]thymidine; Ia, I region-associated antigens; Nle, norleucine, L-2-aminoheptanoic acid; PEL, peritoneal exudate lymphocyte; Phe(4-NH₂), 4-aminophenylalanine; Phe(4-NO₂), 4-nitrophenylalanine; PPD, purified protein derivative of tuberculin; Sar, sarcosine, N-methylglycine.

Materials and Methods

Animals. Inbred strain 2 and strain 13 guinea pigs were obtained from Biological Systems, Toms River, N. J.

Antigens. Synthetic human angiotensin II (AII), [Val⁶]-AII, [Asn¹,Val⁵]-AII, angiotensin III (AIII), [Val⁴]-AIII, [Ile⁷]-AIII, angiotensin I (AI), [Sar¹,Ala⁸]-AII, [Sar¹,Gly⁸]-AII, [Sar¹,Ile⁸]-AII, and [Sar¹,Leu⁸]-AII were purchased from Vega-Fox Biochemicals Div., Newberg Energy Corp., Tucson, Ariz. The analogues [AcAsn¹,Val⁵,des-Phe⁸]-AII, [Asn¹,Phe(4-NH₂)⁴]-AII, [Asn¹,Phe(4-NH₂)⁶]-AII, [Asn¹,Phe(4-NH₂)⁸]-AII, [Asn¹,Phe(4-NO₂)⁴]-AII, [Asn¹,Phe(4-NO₂)⁸]-AII, [Asn¹,Nle⁸]-AII, and [Asn¹,Ahp⁸]-AII, were synthesized and purified as described elsewhere (6-8). The primary structure of each peptide is shown in Fig. 1. The abbreviations used to denote amino acids and peptides are those recommended by the International Union of Pure and Applied Chemistry/International Union of Biochemistry Commission on Biochemical Nomenclature (9).

Preparation of Cells. 2-6 wk after immunization with 400 µg of AII or of the various homologues or analogues in complete Freund's adjuvant (CFA; Difco Laboratories, Detroit, Mich.), guinea pigs were injected intraperitoneally with 25 ml of sterile mineral oil (Marcol 52; Humble Oil and Refining Co., Houston, Tex.), and the resulting peritoneal exudate was harvested 3-4 d later. A T lymphocyte-enriched peritoneal exudate lymphocyte (PEL) population was prepared by passing cells over a rayon wool adherence column (10).

In Vitro Assay of DNA Synthesis. Immune PEL (3-4 × 10⁵ cells/well) were cultured in round-bottom microtiter plates (Cooke Laboratory Products Div., Dynatech Laboratories, Inc., Arlington, Va.) with soluble AII peptide antigens (5-40 µg/well) or purified protein derivative of tuberculin (PPD) (10 µg/well), in a total volume of 0.2 ml of RPMI-1640 medium containing L-glutamine (300 µg/ml), penicillin (100 U/ml), 2-mercaptoethanol (5 × 10⁻⁵ M), and 5% heat-inactivated normal guinea pig serum. After incubation for 2 d at 37°C in 5% CO₂ in air, 1 µCi of tritiated thymidine ([³H]TdR, sp ac 6.7 Ci/mM; Research Products International Corp., Elk Grove Village, Ill.) was added to each well. The amount of radioactivity incorporated into cellular DNA was determined after an additional 18-h incubation with the aid of a semiautomated microharvesting device (Titer-Tek, Flow Laboratories, Inc., Rockville, Md.). Each [³H]TdR cpm value represents the mean of triplicate cultures and the standard error was always within 10% of the mean. The representative experiments shown were each performed 2-6 times with similar results, and 40 µg of the peptide antigens was the dose producing the highest proliferative responses.

Results

Strain 2 and strain 13 guinea pigs were immunized with AII or related analogues and in vitro T cell proliferative responses were determined with the homologous immunizing antigen as well as with other selected AII analogues. A summary of relative strain 2 and strain 13 T cell responsiveness, along with the primary sequence of AII and each analogue used as antigen, is shown in Fig. 1. Most of these results will be detailed in the tables. T cells from strain 2 animals immunized with AII were stimulated by in vitro culture with AII and showed little or no responsiveness with [Val⁵]-AII (Table I). In contrast, T cells from strain 13 guinea pigs immunized with AII were unresponsive with both AII and [Val⁵]-AII. Reciprocal results were obtained after immunization with [Val⁵]-AII: immune strain 13 T cells were stimulated with [Val⁵]-AII but not with AII, and immune strain 2 T cells showed a low response with [Val⁵]-AII and no response with AII. These results indicate that Ir gene control of responsiveness to AII and [Val⁵]-AII by strain 2 and strain 13 guinea pigs is specific and can discriminate between Ile⁵ and Val⁵ residues, which differ only by a methyl group.

The contribution of peptide length to T cell responsiveness was examined by immunization with AI and AIII ([des-Asp¹]-AII) (Table II). AIII is shortened by one

									Immune response	
									Guinea pig	
	H ₂ N-Asp ¹	Arg ²	Val ³	Tyr ⁴	Ile ⁵	His ⁶	Pro ⁷	Phe ⁸ -OH	Strain 2	Strain 13
AII									+++	-
[Val ⁵]-AII					Val				±	+++
[Asn ¹ , Val ⁵]-AII	Asn				Val				-	+++
[Val ⁵]-AIII					Val				-	-
[AcAsn ¹ , Val ⁵ , des-Phe ⁸]-AII	AcAsn				Val				-	+++
AI								His Leu	±	+++
AIII									-	+++
[Ile ⁵]-AIII					Ile				-	-
[Asn ¹ , Phe(4-NH ₂) ⁴]-AII	Asn			Phe(4-NH ₂)					+++	-
[Asn ¹ , Phe(4-NH ₂) ⁶]-AII	Asn					Phe(4-NH ₂)			-	+++
[Asn ¹ , Phe(4-NH ₂) ⁹]-AII	Asn						Phe(4-NH ₂)		-	+++
[Asn ¹ , Phe(4-NO ₂) ⁴]-AII	Asn			Phe(4-NO ₂)					+++	+++
[Asn ¹ , Phe(4-NO ₂) ⁶]-AII	Asn					Phe(4-NO ₂)			+++	+++
[Asn ¹ , Nle ⁶]-AII	Asn					Nle			-	-
[Asn ¹ , Ahp ⁶]-AII	Asn					Ahp			-	-
[Sar ¹ , Ala ⁶]-AII	Sar					Ala			-	-
[Sar ¹ , Gly ⁶]-AII	Sar					Gly			-	-
[Sar ¹ , Ile ⁶]-AII	Sar					Ile			-	-
[Sar ¹ , Leu ⁶]-AII	Sar					Leu			-	-

FIG. 1. Primary structure of AII and related homologues and analogues, and summary of antigenicity and immunogenicity of these peptides for strain 2 and strain 13 guinea pig T lymphocyte responses.

TABLE I
Antigenicity and Immunogenicity of AII and [Val⁵]-AII in Strain 2 and Strain 13 Guinea Pigs

Guinea pig strain	Immunized	[³ H]TdR antigen in culture*			
		None	PPD	AII	[Val ⁵]-AII
			<i>cpm</i>	<i>cpm</i>	
2	AII	1,542 ± 170	<u>119,993 ± 2,577</u>	<u>80,013 ± 2,012</u>	1,464 ± 128
13	AII	6,695 ± 817	<u>120,533 ± 1,774</u>	7,722 ± 104	6,578 ± 268
2	[Val ⁵]-AII	1,385 ± 22	<u>73,299 ± 1,414</u>	1,448 ± 134	6,319 ± 571
13	[Val ⁵]-AII	4,914 ± 310	<u>166,970 ± 2,595</u>	5,534 ± 387	<u>41,190 ± 2,004</u>

* Strain 2 and strain 13 guinea pigs were immunized with AII or AII analogues as indicated. 2-6 wk after immunization, T cell-enriched PEL were cultured in vitro with PPD, as a positive control, and the indicated AII analogues (40 µg/well), as described in Materials and Methods. The [³H]TdR cpm were determined on the 3rd d of culture and each value represents the mean cpm from triplicate cultures ± the standard error. Underlined values indicate those cultures in which positive stimulation occurred; italics indicate those cultures showing relatively less stimulation.

TABLE II
Immunogenicity and Antigenicity of AI and AIII in Strain 2 and Strain 13 Guinea Pigs

Guinea pig strain	Immunized	[³ H]TdR antigen in culture*				
		None	PPD	AII	AI	AIII
			<i>cpm</i>	<i>cpm</i>	<i>cpm</i>	
2	AII	2,627 ± 473	<u>133,767 ± 5,016</u>	<u>36,249 ± 280</u>	4,084 ± 95	4,322 ± 652
2	AI	1,658 ± 74	<u>120,230 ± 6,411</u>	<u>9,302 ± 1,343</u>	<u>3,556 ± 735</u>	1,548 ± 151
2	AIII	2,878 ± 222	<u>176,188 ± 3,539</u>	3,137 ± 272	3,081 ± 189	3,148 ± 332
13	AI	578 ± 172	<u>124,770 ± 1,798</u>	<u>9,489 ± 612</u>	<u>38,007 ± 1,726</u>	<u>3,879 ± 409</u>
13	AIII	616 ± 61	<u>162,600 ± 8,012</u>	<u>6,370 ± 408</u>	<u>13,671 ± 596</u>	<u>43,841 ± 466</u>

* Same as Table I.

residue at the amino terminus, and AI is lengthened by two residues at the carboxyl terminus. Strain 2 AII-immune T cells showed little or no responsiveness with AI and AIII, which indicates that despite considerable residue homology, addition or removal of residues from AII dramatically altered antigenicity. Moreover, these alterations eliminated immunogenicity in strain 2 guinea pigs. Strain 2 animals immunized with AI produced a marginal T cell response with AI, no response with AIII, and showed a relatively low response with AII. T cells from strain 2 guinea pigs immunized with AIII were unresponsive to AI, AII, and AIII. In contrast, changing the length of AII results in peptides with enhanced immunogenicity in strain 13 guinea pigs. Strain 13 AI-immune T cells responded to AI and showed some cross-reactivity with AII and AIII. T cells from AIII-immune strain 13 animals responded with AIII and showed 15 and 30% cross-reactivity with AII and AI, respectively. These results suggest that genetic control of responsiveness to the AII peptides may involve multiple peptide residues. As shown in Table I, strain 13 guinea pigs responded to [Val⁵]-AII but not to AII, which indicates control of responsiveness by the fifth residue. However, strain 13 animals responded to [Ile⁴]-AIII, as shown in Table II, indicating that in this instance the first AII residue also appeared to control responsiveness.

The contributions of the first and fifth AII residues to strain 13 responsiveness to [Val⁵]-AII were further examined using the analogues [Asn¹,Val⁵]-AII and [Val⁴]-AIII as shown in Table III. T cells from [Val⁵]-AII-immune strain 13 guinea pigs responded to the same extent with [Val⁵]-AII and [Asn¹,Val⁵]-AII, but showed no response with [Val⁴]-AIII. Similarly, T cells from [Asn¹,Val⁵]-AII-immune animals responded in the same way with [Val⁵]-AII and [Asn¹,Val⁵]-AII, but were unresponsive with [Val⁴]-AIII. [Val⁴]-AIII-immune strain 13 guinea pigs showed no response with either [Val⁴]-AIII or [Val⁵]-AII. Therefore, it is clear that the first residue of [Val⁵]-AII is critical for strain 13 responsiveness, but that there may be little specificity associated with this position because Asp¹ and Asn¹ are interchangeable. In addition, the presence or absence of the first residue is involved in determining the control of responsiveness by the fifth residue. Thus, strain 13 animals respond to [Val⁵]-AII but not to [Ile⁵]-AII; however, with the removal of Asp¹ (AIII), the animals now respond to [Ile⁴]-AIII only and not to [Val⁴]-AIII. These results indicate that there is no single residue indicative of Ir gene control, and that each peptide analogue represents a unique antigen, responsiveness to which cannot be predicted.

The contribution of Tyr⁴, His⁶, and Phe⁸ to Ir gene control and T cell responses was examined using two sets of analogues with substitutions for these residues. In the first series, Phe(4-NH₂) was substituted for Tyr⁴, His⁶, or Phe⁸, and each analogue was tested for antigenicity and immunogenicity in strain 2 and strain 13 guinea pigs (Table IV). T cells from AII-immune strain 2 animals showed no response with

TABLE III
Role of Asp¹ in Strain 13 Guinea Pig Responses to [Val⁵]-AII

Strain 13 guinea pig immunized	³ H]TdR antigen in culture*				
	None	PPD	[Val ⁵]-AII	[Asn ¹ , Val ⁵]-AII	[Val ⁴]-AIII
[Val ⁵]-AII	4,914 ± 310	166,970 ± 2,595	41,190 ± 2,004	46,378 ± 503	3,878 ± 257
[Asn ¹ , Val ⁵]-AII	3,859 ± 106	193,710 ± 6,146	61,370 ± 508	62,269 ± 4,038	5,675 ± 725
[Val ⁴]-AIII	2,193 ± 69	145,108 ± 1,234	3,436 ± 547	ND‡	3,636 ± 323

* Same as Table I.

‡ Not determined.

TABLE IV
Antigenicity and Immunogenicity of AII Analogues in Which Tyr⁴, His⁶, and Phe⁸ are Replaced by Phe(4-NH₂)

Guinea pig strain	Immunized	³ H]TdR antigen in culture*					
		None	PPD	AII	[Asn ¹ , Phe(4-NH ₂) ⁴]-AII	[Asn ¹ , Phe(4-NH ₂) ⁶]-AII	[Asn ¹ , Phe(4-NH ₂) ⁸]-AII
			<i>cpm</i>		<i>cpm</i>		<i>cpm</i>
2	AII	2,627 ± 473	133,767 ± 5,016	36,249 ± 237	3,476 ± 237	2,876 ± 404	2,623 ± 160
2	[Asn ¹ , Phe(4-NH ₂) ⁴]-AII	2,353 ± 297	180,999 ± 3,736	2,160 ± 104	54,451 ± 4,146	2,216 ± 386	3,972 ± 456
2	[Asn ¹ , Phe(4-NH ₂) ⁶]-AII	1,187 ± 188	146,366 ± 7,052	1,244 ± 75	1,041 ± 259	879 ± 34	987 ± 24
2	[Asn ¹ , Phe(4-NH ₂) ⁸]-AII	3,393 ± 69	125,548 ± 964	4,197 ± 429	3,086 ± 1,059	3,282 ± 246	3,184 ± 461
13	[Asn ¹ , Phe(4-NH ₂) ⁴]-AII	800 ± 87	117,428 ± 4,983	1,219 ± 109	1,566 ± 203	902 ± 173	1,359 ± 236
13	[Asn ¹ , Phe(4-NH ₂) ⁶]-AII	3,376 ± 129	262,600 ± 12,059	5,304 ± 560	3,185 ± 340	47,345 ± 1,545	80,295 ± 13,420
13	[Asn ¹ , Phe(4-NH ₂) ⁸]-AII	1,505 ± 52	158,177 ± 19,841	9,256 ± 338	ND‡	15,696 ± 290	31,239 ± 1,415

* Same as Table I.

‡ Not determined.

TABLE V
Antigenicity and Immunogenicity of AII Analogues in Which Tyr⁴ and Phe⁸ are Replaced by Phe(4-NO₂)

Guinea pig strain	Immunized	³ H]TdR antigen in culture*				
		None	PPD	AII	[Asn ¹ , Phe(4-NO ₂) ⁴]-AII	[Asn ¹ , Phe(4-NO ₂) ⁸]-AII
			<i>cpm</i>		<i>cpm</i>	<i>cpm</i>
2	AII	841 ± 61	122,786 ± 1,416	21,026 ± 401	812 ± 127	850 ± 105
2	[Asn ¹ , Phe(4-NO ₂) ⁴]-AII	2,958 ± 41	150,191 ± 9,480	5,720 ± 760	111,630 ± 6,388	23,830 ± 1,005
2	[Asn ¹ , Phe(4-NO ₂) ⁸]-AII	3,184 ± 236	198,244 ± 2,650	30,456 ± 1,363	4,648 ± 627	28,010 ± 653
13	[Asn ¹ , Phe(4-NO ₂) ⁴]-AII	1,549 ± 30	140,908 ± 4,046	2,026 ± 244	53,412 ± 2,771	10,909 ± 336
13	[Asn ¹ , Phe(4-NO ₂) ⁸]-AII	442 ± 37	105,573 ± 2,353	3,196 ± 272	7,677 ± 150	17,142 ± 517

* Same as Table I.

[Asn¹,Phe(4-NH₂)⁴]-AII, [Asn¹,Phe(4-NH₂)⁶]-AII, or [Asn¹,Phe(4-NH₂)⁸]-AII, indicating that these substitutions altered antigenicity. On the other hand, strain 2 T cells immunized with [Asn¹,Phe(4-NH₂)⁴]-AII were responsive with the immunizing analogue but were unresponsive with AII, [Asn¹,Phe(4-NH₂)⁶]-AII, and [Asn¹,Phe(4-NH₂)⁸]-AII. [Asn¹,Phe(4-NH₂)⁶]-AII and [Asn¹,Phe(4-NH₂)⁸]-AII were nonimmunogenic for strain 2 guinea pigs and failed to prime for responsiveness to AII. In contrast, immunization of strain 13 animals with the same analogues produced a reciprocal pattern of responsiveness. [Asn¹,Phe(4-NH₂)⁴]-AII was nonimmunogenic in strain 13, whereas immunization with [Asn¹,Phe(4-NH₂)⁶]-AII and [Asn¹,Phe(4-NH₂)⁸]-AII resulted in good T cell responses. However, T cells from strain 13 animals immunized with these latter two analogues showed considerable cross-reactivity. Some cross-reactivity was also observed with AII after immunization with [Asn²,Phe(4-NH₂)⁸]-AII.

A second set of analogues in which Tyr⁴ and Phe⁸ were substituted with Phe(4-NO₂) instead of Phe(4-NH₂), as above, also produced a distinct pattern of strain 2 and strain 13 T cell responses (Table V). Strain 2 AII-immune T cells were unresponsive with [Asn¹,Phe(4-NO₂)⁴]-AII and [Asn¹,Phe(4-NO₂)⁸]-AII, again indicating that these substitutions altered the antigenicity of AII. T cells from strain 2 animals immunized with [Asn¹,Phe(4-NO₂)⁴]-AII responded with [Asn¹,Phe(4-NO₂)⁴]-AII and showed 20% cross-reactivity with [Asn¹,Phe(4-NO₂)⁸]-AII, but were unresponsive with

AII. [Asn¹,Phe(4-NO₂)⁸]-AII-immune strain 2 T cells were equally responsive with both [Asn¹,Phe(4-NO₂)⁸]-AII and AII, but were unresponsive with [Asn¹,Phe(4-NO₂)⁴]-AII. Immunization of strain 13 guinea pigs with [Asn¹,Phe(4-NO₂)⁴]-AII resulted in T cell responses with [Asn¹,Phe(4-NO₂)⁴]-AII and showed 20% cross-reactivity with [Asn¹,Phe(4-NO₂)⁸]-AII, but were unresponsive with AII. [Asn¹,Phe(4-NO₂)⁸]-AII-immune strain 13 T cells responded with [Asn¹,Phe(4-NO₂)⁸]-AII and produced 45% cross-reactivity with [Asn¹,Phe(4-NO₂)⁴]-AII and 20% cross-reactivity with AII. This pattern of cross-reactivity with AII and [Asn¹,Phe(4-NO₂)⁴]-AII after immunization of strain 13 with [Asn¹,Phe(4-NO₂)⁸]-AII is clearly much different from that obtained in strain 2 guinea pigs. These results indicate that although both strain 2 and strain 13 animals respond to the same octapeptide antigen, [Asn¹,Phe(4-NO₂)⁸]-AII may be recognized differently by strain 2 and strain 13 T cells. It should be noted that T cell responses to Phe(4-NH₂)-containing analogues were non-cross-reactive with Phe(4-NO₂)-containing analogues and vice versa (data not shown).

A number of other AII analogues primarily containing substitutions for Phe⁸ were examined for antigenicity and immunogenicity in strain 2 and strain 13 guinea pigs and were found to be nonimmunogenic (summarized in Fig. 1). These results indicate that the carboxyl terminal residue is important for T cell responses and suggest that aromaticity in this position is required. However, it should be noted that removal of this residue, as in the analogue [AcAsn¹,Val⁵,des-Phe⁸]-AII, restores immunogenicity in strain 13, but not in strain 2 guinea pigs.

Discussion

In this study we have examined the specificity of Ir gene control and T cell recognition of a series of small peptide antigens based on the octapeptide, angiotensin II. A summary of strain 2 and strain 13 guinea pig T cell responses to these antigens is shown in Fig. 1. It is clear that nearly all modifications of AII, either single residue substitutions or alterations in peptide length, have a dramatic effect on Ir gene control and/or T cell recognition. The fact that most of these changes result in all-or-none effects on T cell responses suggests that the overall response is probably restricted to several clones. In addition, the small size of these peptide antigens seems to severely restrict potential immunocompetent cellular interactions. For these reasons, we feel that the observed responses are representative of T cell recognition of antigen presented by stimulator cells and may not involve more complicated regulatory mechanisms. In most of these cases, it appears that Ir gene control and the specificity of T cell responses are linked, but there are situations in which specificity and Ir gene control are to some extent independently coordinated. The discussion below will therefore treat Ir gene control and T cell specificity separately, and then compare both aspects to develop a model for T cell recognition.

Specificity of Ir Gene Control. One of the most striking features of the AII antigen system is the exquisite specificity of Ir gene control of T cell responsiveness, as summarized in Fig. 1. For example, strain 2 guinea pigs respond to AII and show little responsiveness to [Val⁵]-AII, whereas strain 13 animals respond to [Val⁵]-AII and not to AII. Therefore, Ir gene control of responsiveness in this situation is regulated by the single methyl-group difference between Val⁵ and Ile⁵. Based on this observation, it was anticipated that strain 2 animals would respond to any AII analogue containing Ile⁵ and strain 13 would respond to any analogue containing

Val⁵. This was not observed, however, and regulation of responsiveness by the fifth residue in AII also involved the amino terminal residue. Thus, strain 13 guinea pigs failed to respond to [Val⁴]-AIII, but responded to [Ile⁴]-AIII. In this case, the presence or absence of Asp¹ must also be involved in strain 13 responsiveness to AII. These results suggest that no one peptide residue determines Ir gene control, but that genetic control of responsiveness relates to the overall structure of the peptide antigen. This implies that each peptide analogue may represent a distinct antigenic species, responsiveness to which cannot be predicted based on responses to closely related antigens.

The other examples of the specificity of Ir gene control are shown with AII analogues in which Tyr⁴ and Phe⁸ have been substituted with Phe(4-NH₂) or Phe(4-NO₂). Strain 13 guinea pigs respond to [Phe(4-NO₂)⁴]-AII but are unresponsive to [Phe(4-NH₂)⁴]-AII, and strain 2 animals respond to both analogues. On the other hand, strain 2 guinea pigs respond to [Phe(4-NO₂)⁸]-AII and are unresponsive to [Phe(4-NH₂)⁸]-AII, whereas strain 13 animals respond to both analogues. These results provide further evidence that the genetic control of T cell responsiveness shows extremely fine specificity and can discriminate between subtle changes in the AII residues.

Specificity of T Lymphocyte Recognition. In a previous study of T cell responses to human fibrinopeptide B and closely related analogues, we found that several residues determined the clonal specificity of T cell responses (3). Similarly, with AII we found residues that seemed to be critical for the specificity of T cell responses. In strain 2 guinea pigs, for example, substitutions of Phe(4-NH₂) and Phe(4-NO₂) for Tyr⁴ resulted in analogues that elicited unique non-cross-reactive T cell responses, indicating that Tyr⁴ may make a major contribution to the specificity of antigen recognition. A similar analysis in strain 13 animals cannot be made because these same substitutions determined responsiveness or unresponsiveness. However, substitutions of Phe(4-NH₂) or Phe(4-NO₂) for Phe⁸ resulted in unique non-cross-reactive T cell responses in strain 13 animals. Again, a similar analysis cannot be made in strain 2 animals because these substitutions determine responsiveness. As discussed previously, Asp¹ does not seem to be involved in specificity because Asn¹ is interchangeable with Asp¹. Thus, the specificity of T cell responses can be altered by substitutions for Tyr⁴, His⁶ (in strain 13), and Phe⁸.

Comparisons of Ir Gene Control and T Lymphocyte Recognition. It is clear from the preceding discussion that Ir gene control of T cell responses to the AII antigens is highly specific and may involve multiple interactions with the peptide. Moreover, several residues were identified that seemed to be important for both the specificity and genetic control of T cell responses. These observations suggest that Ir gene control and T cell recognition of antigen may be intimately associated. One of the more striking observations derived from the antigen survey shown in Fig. 1 is the pattern of strain 2 and strain 13 responses. In general, strain 2 and strain 13 animals do not respond to the same peptide antigens. In fact, out of 37 peptide antigens we have examined thus far in several systems, strain 2 and strain 13 guinea pigs rarely responded to the same peptide. The exceptions to this observation are T cell responses to [Phe(4-NO₂)⁴]-AII and [Phe(4-NO₂)⁸]-AII. However, [Phe(4-NO₂)⁸]-AII-immune strain 2 T cells show total cross-reactivity with AII and are unresponsive with [Phe(4-NO₂)⁴]-AII, whereas immune strain 13 T cells show the reciprocal pattern of cross-reactivity. Therefore, although both strain 2 and strain 13 animals respond to [Phe(4-

NO₂)⁹]-AII, the antigen is probably recognized differently. These results, although limited, suggest that the expression of the antigen-combining repertoire of strain 2 and strain 13 T cells is generally nonoverlapping. This implies that T cells from these two strains rarely recognize an antigen in precisely the same manner. This difference may never be seen using larger proteins that contain a variety of antigenic determinants, but it is obvious when examining responses to small peptides that limit the number of available antigenic determinants. This point is of considerable importance and will be considered later in developing a model for T cell recognition.

The other important point made in this survey is that there are many "holes" in T cell responses to closely related peptide analogues. In many of these cases responsiveness fluctuates between strain 2 and strain 13 guinea pigs. However, the residues regulating responsiveness may serve more than one function. For example, in strain 2 guinea pigs substitutions for Tyr⁴ alter the specificity of the T cell response, whereas in strain 13 they determine responsiveness or unresponsiveness. Similarly, substitutions for Phe⁸ determine the specificity of strain 13 T cell responses, whereas in strain 2 they determine responsiveness. Thus, there are situations in which residue substitutions alter the specificity of the T cell response without changing Ir gene control (3), and situations where specificity and Ir gene control are both changed. These observations may be due to the apparent nonoverlap of strain 2 and strain 13 T cell antigen recognition, because each residue may serve a different function in responses by both strains.

Comparisons of T Lymphocyte Responses with Antibody and Hormone Activity of AII. As indicated in the introduction, angiotensin has been widely used to examine antibody binding using the same approach employed here to investigate T cell responses. In addition, a number of studies have been performed to determine the parameters of AII binding to the hormone receptor (11). It is therefore of interest to compare our findings concerning T cell responses with those measuring AII reactivity using these other types of receptors. Briefly, AII binding by rabbit anti-AII antibody was substantially reduced only by residue substitutions for Phe⁸, Pro⁷, Tyr⁴, and to a lesser extent His⁶ (4). For biological activity, the most important residues were Phe⁸, Pro⁷, His⁶, Tyr⁴, and Arg² (11). It is clear from these analyses that guinea pig T cell responses are dependent on two residues that are less critical for antibody or hormone receptor binding, Asp¹ and Ile⁵. This suggests that AII recognition by guinea pig T cells is different from AII binding by rabbit antibody or the hormone receptor.

Implications for Antigen Binding by T Cells. The observation that T cell recognition of AII seems to be more complex than other highly specific AII receptors implies that the antigen combining site for AII in T cell responses must be somewhat rigid, with well-defined spatial and contact parameters. The formation of such a receptor must also take into account the observations for Ir gene control, which indicate that the antigen-combining repertoire of strain 2 and strain 13 T cells appears to be generally exclusive. As demonstrated previously, the differences in the genetic control of immune responses are determined by the expression of macrophagelike stimulator cell Ia antigens (2, 12-16). Therefore, considerations for antigen recognition in T cell responses must take into account both the antigen-combining capacity of T cells, presumably effected through a clonally distributed receptor, and the involvement of stimulator cell Ia antigens. The simplest explanation would be that the difference in strain 2 and strain 13 T cell recognition for various AII antigens is based solely in the

T cell antigen-combining receptor. Thus, the genetic basis for T cell diversity would be due to different variable-like regions in strain 2 and strain 13 T cell receptors. In this case, Ia may serve only as a second signal to affect T cell activation, and/or Ia may serve to select particular variable-like regions expressed by T cells. However, it is difficult to imagine that the variable-like region genes would be exclusive in strain 2 and strain 13 T cells, or that Ia could "pick out" closely related variable-like region genes to be expressed, particularly in view of the fine antigenic distinctions demonstrated in anti-AII T cell responses.

A more likely possibility is that the repertoire of variable-like region genes for T cell receptors is similar in strain 2 and strain 13 guinea pigs and that stimulator cell Ia antigens are involved in the specificity of the response at the time of antigen exposure. Again, the specificity of Ir gene control strongly suggests an intimate involvement of Ia antigens in the recognition of exogenous antigens, probably in conjunction with the antigen-combining T cell receptor. There are two types of models that would accommodate all of the observations made here. The first is the determinant selection model proposed by Rosenthal (17) and Benacerraf (18). According to this model, Ia antigens show discriminating antigen-combining properties and bind antigen before interaction with T cells. Because different Ia molecular species bind different antigenic determinants, distinct determinants are available for T cell recognition. Based on the data presented here, this model would suggest that Ia antigens show very fine antigen-combining properties that could distinguish Val⁵ from Ile⁵, for example. In addition, we observed nine situations in which genetic control fluctuated between strain 2 and strain 13 T cell responses to closely related peptides, which, according to this model, would imply a minimum of nine different Ia molecular species for the AII antigens tested thus far. Although biochemical analyses have indicated that guinea pig Ia antigens are fairly homogeneous (19), complete residue sequences will be required to establish their degree of heterogeneity.

A second model, as we proposed before (3), is that the antigen-combining site is formed by the physical interaction between the T cell receptor and stimulator cell Ia antigens, similar to heavy and light chain interactions in immunoglobulins. This Ia-T cell receptor interaction defines the spatial and contact parameters within the newly formed antigen-combining site and thus creates the specificity of T cell recognition and Ir gene control. Much of the contact specificity would be contributed by clonally expressed T cell receptors, whereas stimulator cell Ia antigens may primarily stabilize the T cell receptor and determine the spatial constraints of the combining site. Ia molecules might also provide some limited contact sites for antigen, but this may depend on the particular antigen and how it is accommodated in the combining site. In contrast to the determinant selection model, this model predicts that antigen will only be bound after stabilization of the combining site by the Ia-T cell receptor interaction.

Both models suggest that the basis for genetic restrictions between T cells and stimulator cells is simply clonal selection for T cells with different antigen-combining properties, similar to selecting T cells that distinguish ovalbumin from human gammaglobulin, and that there is no inherent Ia restriction distinct from antigen recognition. In addition, the final recognition complex, once formed, would be identical according to both models. However, it may be possible to distinguish between these models experimentally by using the AII antigen system described here.

One prediction of the determinant selection proposal is that strain 2 and strain 13 Ia molecules would contact the residue determining genetic control, e.g., strain 2 Ia contacts Ile⁵ and strain 13 Ia contacts Val⁵, thus leaving similar antigenic determinants free to interact with strain 2 and strain 13 T cells. Thus, T cells from (2 × 13)F₁ animals immunized with AII would be expected to respond not only to AII in association with strain 2 stimulator cells, but also with [Val⁵]-AII in association with strain 13 stimulator cells. According to our alternative model, AII-immune (2 × 13)F₁ T cells would respond only to AII associated with strain 2 stimulator cells, and not to any AII analogue or homologue with strain 13 stimulator cells, because much of the specificity of I_r gene control would reside with the T cell antigen combining receptor in this case. These and related experiments should provide useful information toward our understanding of T cell recognition of antigen and the role of Ia antigens in this process.

Summary

Guinea pig T lymphocyte responses to the octapeptide antigen angiotensin II (NH₂-Asp¹-Arg²-Val³-Tyr⁴-Ile⁵-His⁶-Pro⁷-Phe⁸-OH; AII) were examined using various synthetic peptide analogues and homologues. Each peptide antigen was assessed for immunogenicity and antigenicity in strain 2 and strain 13 guinea pigs as determined by in vitro T cell proliferative responses. The genetic control of T cell responses to these peptides was found to be highly specific and capable of distinguishing subtle differences in the antigens. For example, strain 2 guinea pigs responded to AII and were low responders to [Val⁵]-AII, whereas strain 13 animals responded to [Val⁵]-AII but not to AII. The genetic control in this case involved the difference of one methyl group between Val⁵ and Ile⁵. Differences in T cell responsiveness by strain 2 and strain 13 guinea pigs were also observed with analogues involving para substitutions on the phenyl ring of Tyr⁴ and of Phe⁸. However, the genetic regulation of T cell responses did not seem to be based on a single peptide residue. For example, removal of Asp¹ allowed strain 13 animals to respond to the Ile⁵-containing analogue, but eliminated responsiveness to the Val⁵-containing analogue. Thus, the first and fifth AII residues are both involved in the regulation of strain 13 T cell responses. Substitutions for Tyr⁴ and Phe⁸ suggested that the same residue may serve to alter the specificity of T cell responses in one strain, and determine responsiveness or unresponsiveness in the other strain. One of the most striking observations is that T cell responsiveness to the various AII analogues and homologues randomly fluctuates between strain 2 and strain 13 guinea pigs, and in general neither strain responds to the same peptide antigens. This suggests that strain 2 and strain 13 T cell responses are rarely directed against the same antigenic determinants, and that the T cell antigen-combining diversity is usually exclusive between these two strains. These results are discussed with respect to the specificity of I_r gene control and the relationship between I_r gene function and antigen recognition by T cells.

Note added in proof: More recent experiments using a new lot of [Val⁵]-AII have indicated that [Val⁵]-AII-immune strain 2 T cells show significant stimulation with AII but remain relatively low responders with [Val⁵]-AII, as shown in Table I. The

difference in priming for cross-reactivity for AII with the different lots of [Val⁵]-AII is at present unknown.

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