Regulation of the Immune Response to Peptide Antigens: Differential Induction of Immediate-type Hypersensitivity and T Cell Proliferation Due to Changes in Either Peptide Structure or Major Histocompatibility Complex Haplotype

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Summary

The immunodominant CD4 T cell epitope of the bacteriophage λ cI repressor protein in several inbred mouse strains can be represented by a peptide encompassing amino acids 12–26. Here, we show that this peptide, and a variety of its sequence variants, can induce immediate-type hypersensitivity in mice. 12–26 variants that differ by as little as single amino acid residues deviate greatly in their ability to induce hypersensitivity. Further, differences in major histocompatibility complex class II alleles appear to be as influential as changes in peptide structure in determining whether hypersensitivity is developed. The ability of a given peptide–class II combination to induce hypersensitivity correlates with production of peptide-specific antibody, but not with ability or inability to induce a T cell proliferative response. Administration of anti–interleukin 4 (IL-4) mAb prevents the development of hypersensitivity, and analysis of cytokine production by T cell hybridomas derived from peptide-immunized mice suggests that whether a given peptide–class II combination can induce hypersensitivity depends on its ability to induce IL-4 production. The data demonstrate that changes in the nature of the epitope(s) recognized by the CD4 T cell population can result in qualitative differences in the response elicited in this population, ultimately leading to dramatic quantitative and qualitative variations in the effector phase of the immune response.

The ligand for most CD4 TCRs of the α/β type is a complex of a class II MHC molecule and a peptide subfragment of a protein antigen. This ligand is created on the surface of an APC via internalization of the antigen, its degradation to peptide fragments, and association of some of these fragments with class II molecules via agretypic determinants on the peptides (1). Linear peptides of 5–20 amino acids bearing agretypic and T cell epitypic determinants can substitute for intact antigen in in vitro T cell activation assays (2–4). These findings have been exploited towards the development of synthetic peptide vaccines (5, 6), and the design of peptides that can block autoimmune T cell responses in vivo (7, 8).

Linear synthetic peptides are much simpler in structure than the high molecular weight protein antigens normally used to elicit immune responses. In addition, synthetic peptide chemistry allows facile generation of an assortment of different mutant forms of any given peptide. Thus, changes in putative epitypic and agretypic determinants in a peptide can easily be made and assayed for their effect on immunogenicity in vitro and in vivo. Studies from a number of laboratories have demonstrated the utility of this approach towards the elucidation of the structural correlates of T cell immunogenicity (9–11). Such studies have shown that changes in the primary structure of synthetic peptides often result in alterations in their immunogenicity that are not easily explained by our current understanding of the nature of agretypic and epitypic determinants (12–14).

While the CD4 T cell immune response to linear synthetic peptides is an active area of research, less attention has been paid to the B cell (antibody) response to such antigens. If a peptide contains an agretope and B and CD4 T cell epitopes, it might be expected to elicit a conventional humoral response in vivo. Indeed, a variety of short synthetic peptides have been shown to be capable of inducing vigorous

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antibody responses (15–17). Therefore, linear synthetic peptides should prove useful as chemically defined model antigens for the elucidation of the structural correlates of humoral immunogenicity. In theory, independent changes in the components currently known to be essential for humoral immunogenicity, namely, B cell and CD4 T cell epitypic as well as agretypic determinants, could be made in a given peptide and assayed for their influence on the quantitative and qualitative outcome of the antibody response to the peptide.

To initially investigate how changes in the structure of a linear synthetic peptide antigen might influence its humoral immunogenicity, we chose to study the immune response to synthetic peptides representing an immunodominant CD4 T cell epitope of the bacteriophage λ cI repressor. A large fraction of the CD4 T cells that respond to the λ cI repressor in BALB/c and A/J mice are specific for the 12–26 region (18, 19) as defined by in vitro T cell activation assays using a synthetic peptide encompassing these residues.

Materials and Methods

Peptide Synthesis and Purification. Peptides were synthesized as described (20) using a peptide synthesizer (430A; Applied Biosystems, Inc., Foster City, CA), and purified via either reverse-phase HPLC or a combination of HPLC and ion exchange chromatography. All peptides were sequenced using a protein sequencer (470A; Applied Biosystems, Inc.) before use. Proton-nuclear magnetic resonance (NMR)[1] spectra and amino acid compositions were obtained for selected peptides and demonstrated that they were >95% pure.

Immunization of Mice and Assay of Hypersensitive Responses and Serum Antipeptide Antibody. Lyophilized peptides were dissolved in PBS at 2 mg/ml and then emulsified in CFA at a ratio of one volume of adjuvant to one volume of peptide solution. Mice were immunized with 100 μl of this emulsion intraperitoneally. Both IFA and Alum (a 9% solution mixed 1:1 with the antigen solution) were also used as adjuvants. 3 wk after immunization, 150-μl blood samples were taken. 1 mo or more after priming, the mice were boosted with 100 μg of peptide in either PBS, IFA, or Alum, intraperitoneally. The mice were then observed for at least 1 h to evaluate symptoms of hypersensitivity (see text). In peptide-mouse strain combinations that did not display hypersensitivity, symptoms were not observed even if the boosting dose of peptide was raised to 200 μg, or if mice were challenged multiple times at 2-wk intervals. Peptide-specific IgE was assayed using a rat passive cutaneous anaphylaxis (PCA) assay (21). Halothane was used to anesthetize rats, rats were sensitized with 100 μl of a 1:10 dilution of serum or greater injected intradermally on the back, rested for at least 2 h, and challenged with 300 μg of peptide in 300 μl of PBS/0.5% Evans's blue injected into the tail vein. κ light chain–bearing peptide-specific antibody was assayed using a solid phase RIA (22). The heavy chain isotypes of serum antibodies specific for each peptide were also evaluated using a solid phase RIA (22). A set of affinity-matched antiarsonate mAbs representing the different isotypes (see reference 22 for details) were used as controls. The values of serum dilutions that gave half-maximal binding to the peptide-BSA plates were then used to calculate the isotype values cited in the text, after correction for the different binding capacities and avidities of the rabbit antiisotype sera. Peptides were covalently crosslinked to BSA using carbodiimide as described (23). Histological examinations were performed on formalin-fixed tissues by Anmed Biosafe (Rockville, MD).

T Cell Proliferation Assays. Groups of at least four A/J mice were immunized in both hind footpads and at the base of the tail with 100 μg of each peptide emulsified in CFA. 1 wk later, inguinal, popliteal, and para-aortic lymph nodes (LN) were taken, and pooled single cell suspensions were generated. 100-μl microcultures were created using 5×10^5 LN cells and either different concentrations of the peptide used for immunization or no additive. Cultures were incubated for 2 d at 37°C, and then 1 μCi of [^{3}H]thymidine (35 Ci/mmol) was added. The cultures were incubated for at least a further 6 h, and the cells harvested on glass fiber filters. The filters were dried, and incorporated ^{3}H measured by scintillation counting.

Generation of T Cell Hybridomas and Cytokine Assays. Groups of four A/J mice were immunized with 100 μg of peptide, and either 7 or 30 d later, spleens were taken, single cell suspensions prepared, stimulated with 10 μg/ml peptide in vitro, fused to BW5147$\alpha^-\beta^-$ (24), and hybridomas selected, all as previously described (19). The resulting hybridomas were then stimulated with 10 μM of the immunizing peptide using the TA3 lymphoma as APC. After 1 d, supernatants were harvested and IL-2 and IL-4 were assayed using the CTLL.2 and CT.4S (25) indicator lines, respectively. The 11B11 anti-IL-4 mAb (26) was included in CTLL.2 cultures to prevent overlap stimulation by IL-4. A cytokine response to peptide judged to be significant gave indicator line proliferation of at least 10-fold above controls lacking peptide. Such a response corresponded to that induced by 0.5 U/ml of recombinant cytokine (Genzyme, Boston, MA). The CTLL response to IL-2 was reduced by 15% when grown in media-containing 10% (volume basis) of 11B11 hybridoma supernatant. An IL-4 response under such conditions was undetectable.

Results

Synthetic Peptides Representing the Immunodominant CD4 T Cell Epitope of the Bacteriophage λ cI Repressor Protein Induce Immediate-type Hypersensitivity in Mice. Previous experiments have shown that a linear synthetic peptide encompassing the 12-26 region of cI repressor can prime a CD4 T cell response and elicit a serum antibody response in BALB/c mice (27). In these previous experiments, 12-26 was administered in Freund's adjuvant to elicit both primary (CFA) and secondary (IFA) responses. However, when BALB/c mice were given a secondary intraperitoneal challenge of 100 μg of 12-26 in saline 1 mo after a primary intraperitoneal injection of 100 μg of 12-26 in CFA, a major fraction of the mice died within 1 h. Death was preceded by a cumulative progression of the following symptoms: reddening of the ears, tail, and footpads; lack of movement upon prompting; and shallow breathing and prostrate posture. Necropsy revealed severe reddening of the intestines and lungs. Mice that did not die displayed many of these symptoms before an apparent complete recovery ~2 h after injection. The nature of these symptoms as well as their kinetics suggested systemic anaphylaxis, a diagnosis that was supported by histopathology of tissue sections obtained from the lungs, heart, and liver, which revealed extensive vascular congestion.

[1] *Abbreviations used in this paper:* LN, lymph node; NMR nuclear magnetic resonance; PCA, passive cutaneous anaphylaxis.

The severity of symptoms observed upon secondary challenge was dependent on both the primary and secondary dose of peptide, as well as the method of administration. 50 μg of peptide was the smallest amount that could be given either at primary or secondary injection if reproducible symptoms were to be observed. Primary immunization with peptide in saline or IFA did not result in sensitization, while the use of Alum yielded a very low level of hypersensitivity. Secondary challenge with peptide in IFA or on Alum did not result in a hypersensitive reaction. Secondary challenge could be given either intraperitoneally or intravenously, with intravenous injection resulting in a slightly more rapid development of symptoms. Secondary injection in the footpad led to rapid local swelling that was often followed by systemic anaphylaxis.

To rule out the possibility that contaminants in the 12-26 peptide preparation, or in preparations of peptides used in subsequent analyses, could be responsible for the induction of hypersensitivity, three approaches were taken: (*a*) two independent preparations of each peptide were used in most cases and yielded similar results; (*b*) amino acid sequencing, amino acid composition, and proton NMR analyses were done on many of the purified peptide preparations and revealed all to be >95% pure; and (*c*) a peptide with the same amino acid composition as a variant 12-26 peptide (12-26F22Y27; see below) but of a "random" sequence was used for immunization and found to fail to induce hypersensitivity. Collectively, these investigations revealed that the induction of hypersensitivity is not due to contaminants that co-purify with the peptides.

12-26-based Peptide Induction of Hypersensitivity Is MHC Restricted and CD4 T Cell Dependent. Further investigations of this phenomenon showed that it was not confined to BALB/c mice and the 12-26 peptide, but could be observed in a variety of different strains of mice using either 12-26 or amino acid variants of 12-26. Table 1 summarizes these results. The induction of hypersensitivity is CD4 T cell dependent, since hypersensitivity is induced by 12-26F22Y27 in BALB/c mice but not in athymic BALB/c *nu/nu* mice, and treatment of A/J mice with a mAb (GK1.5) specific for the CD4 cell surface antigen (28) before and during the primary anti-12-26F22Y27 response protects them from a hypersensitivity reaction upon secondary challenge. While most of the 12-26-based peptides induced hypersensitivity in BALB/c and C.AL-20 mice (a BALB/c-derived congenic line that bears the $IgH1^d$ locus), several peptides failed to induce hypersensitivity in A/J mice.

Use of other inbred strains and strain A congenic mice differing only in subregions of the MHC revealed that the induction of hypersensitivity by the 12-26F22Y27 peptide is MHC restricted, requiring the presence of class II MHC alleles ($I\text{-}A^d$ or $I\text{-}E^k$) previously shown to encode restricting elements for the 12-26 region of cI repressor (18, 29). In Table 2, such alleles are underlined. The severity of hypersensitivity reactions seems to be affected by factors other than MHC antigens, however. Strains that bore the b alleles of I-A and I-E did not develop hypersensitivity, consistent with previous observations that $H2^b$ mice are T cell nonresponders to the 12-26 region of cI repressor (27). Taken together with the data presented in Table 2, the data shown in Table 1 indicate

Table 1. *Hypersensitivity Responses to Various Peptides by Mice of Different Inbred Strains*

Peptide	Sequence	Hypersensitive Response		
		A/J	BALB/c	C.AL-20
cI Repressor	. . . . Q E Q L E D A R R L K A I Y E K K K N E L			
12-26	- - - - - - - - - - - - - - - -	No	Yes	Yes
12-26F22Y27	- - - - - - - - - - F - - - - Y	Yes	Yes	Yes
12-26F22	- - - - - - - - - - F - - - -	No	Yes	Yes
12-26Y27	- - - - - - - - - - - - - - - - Y	No	No	No
12-26F27	- - - - - - - - - - - - - - - - F	Yes	Yes	Yes
12-24F22	- - - - - - - - - - F - -	No	Yes	ND
12-26C11F22Y27	C - - - - - - - - - - F - - - - Y	Yes	ND	ND
9-29	- -	Yes	Yes	ND
9-29Ac	A c -	Yes	ND	Yes
Random 12-26F22Y27	D I L K Y K R K A F E K L E A R	No	ND	ND

Shown are the name designation of each peptide, its amino acid sequence as compared to the cI repressor using the one-letter code, and whether the peptide induces hypersensitivity in three strains of mice. Dashes indicate sequence identity. Differences are shown explicitly. C.AL-20 is a congenic strain bearing the IgH locus of A.LN ($IgH1^d$) on a BALB/c background. The 9-29Ac peptide has an acetylated NH_2 terminus. In most cases, the data represent the sum of two independent experiments using different preparations of each peptide.

Table 2. *Hypersensitive Responses of Mice with Different MHC Haplotypes to the Peptide 12-26F22Y27*

Strain		H2 K	H2 I-A	H2 I-E	H2 S	H2 D	Hypersensitive response to 12-26F22Y27
Inbred	A/J	k	k	k	d	d	+ + +
	C3H	k	k	k	k	k	+*
	CBA/J	k	k	k	k	k	+ +
	CBA/NJ	k	k	k	k	k	+ + + +
	BALB/c	d	d	d	d	d	+ +
	C.AL-20	d	d	d	d	d	+ + + +
	C57BL/6	b	b	b	b	b	–
	C57BL/10	b	b	b	b	b	–
	CAF1	k/d	k/d	k/d	d	d	+ + +
	A/WySn	k	k	k	d	d	+ +
MHC congenic	A.TL/SfDuEg	s	k	k	k	d	+ + +
	A.TBR1	s	k	k	k	b	+*
	A.TBR16	s	k	k	k	b	+ + +
	A.TBR2	s	k	b	b	b	–
	A.TBR3	s	k	b	b	b	–
	A.TH/SfDuEg	s	s	s	s	d	–
	A.BTR4	b	b	b	b	d	–
	A.BY/Sn	b	b	b	b	b	–
	A.CA/Sn	f	f	f	f	f	–
	B10.A	k	k	k	d	d	+

At least five mice of each strain were immunized and challenged with the 12-26F22Y27 peptide and evaluated for hypersensitivity as described in Materials and Methods. The MHC alleles present in each strain are indicated (H2). Class II MHC alleles (I-A and I-E) previously shown to encode restricting elements for the 12-26 region of cI repressor (18, 29) are underlined. Hypersensitivity reactions were rank ordered as follows: +, reddening of the feet, tail, and ears within 10 min of challenge; + +, visible behavior modification within 15 min of challenge (usually manifest as infrequent movement); + + +, lack of movement upon prompting within 30 min of challenge (prostrate posture); + + + +, death within 2 h after challenge. For each condition, the "scores" of individual mice were averaged. In the case of some strains of mice, only mild symptoms or no symptoms were observed after initial boosting. In these cases, mice were challenged again 2 wk after the initial challenge and re-evaluated for symptoms. Mice that did not display hypersensitivity did not do so even after two challenges of 200 μg of peptide spaced at 2-wk intervals. The origin and characteristics of the A.TBR and A.BTR MHC recombinant strains can be found in references 64 and 65. The data from the congenic mice suggest that the hypersensitive response to 12-26F22Y27 can be I-E^k restricted. In addition, since the s, b, and f haplotypes do not encode a functional I-E molecule (64), the combined data in this table do not rule out the possibility that a hypersensitive response to 12-26F22Y27 requires the I-E molecule in other haplotypes.

* Slightly less than one '+'.

that the $H2^d$ haplotype is more "permissive" than $H2^k$ in allowing induction of hypersensitivity to variant forms of the 12-26 peptide.

Quantitative Differences in the CD4 T Cell Response to Different Peptides. The results presented above suggest that only certain peptide–class II antigen combinations induce the development of a hypersensitive response. These data also show that the presence of an appropriate class II–restricting element(s) for the 12-26 region is not sufficient to allow induction of hypersensitivity by all of the 12-26-based peptides. Before this study, CD4 T cell responsiveness to 12-26 and several of the sequence variants used here had been defined by ability to induce T cell activation in vitro using either T cells that had been primed in vivo using the 1–102 fragment of cI repressor, or T cell hybridomas that had been elicited using this same antigen. In addition, the immunogenicity of several of the variant forms of 12-26 (e.g., 12-26F22Y27) had not been tested in these assays. Therefore, it was possible that mice that did not develop hypersensitivity after immunization with a given 12-26-based peptide might simply be incapable of mounting a CD4 T cell response to that peptide (i.e., were nonresponders). To test this idea, bulk LN T cell stimulation assays were performed. As shown in Fig. 1, these analyses revealed that some of the peptide-strain combinations that did not show evidence of hypersensitivity gave rise to T cell proliferative responses (e.g., 12-26F22 and A/J). Therefore, mice can be T cell responders to a peptide without developing hypersensitivity to that peptide. Moreover, a com-

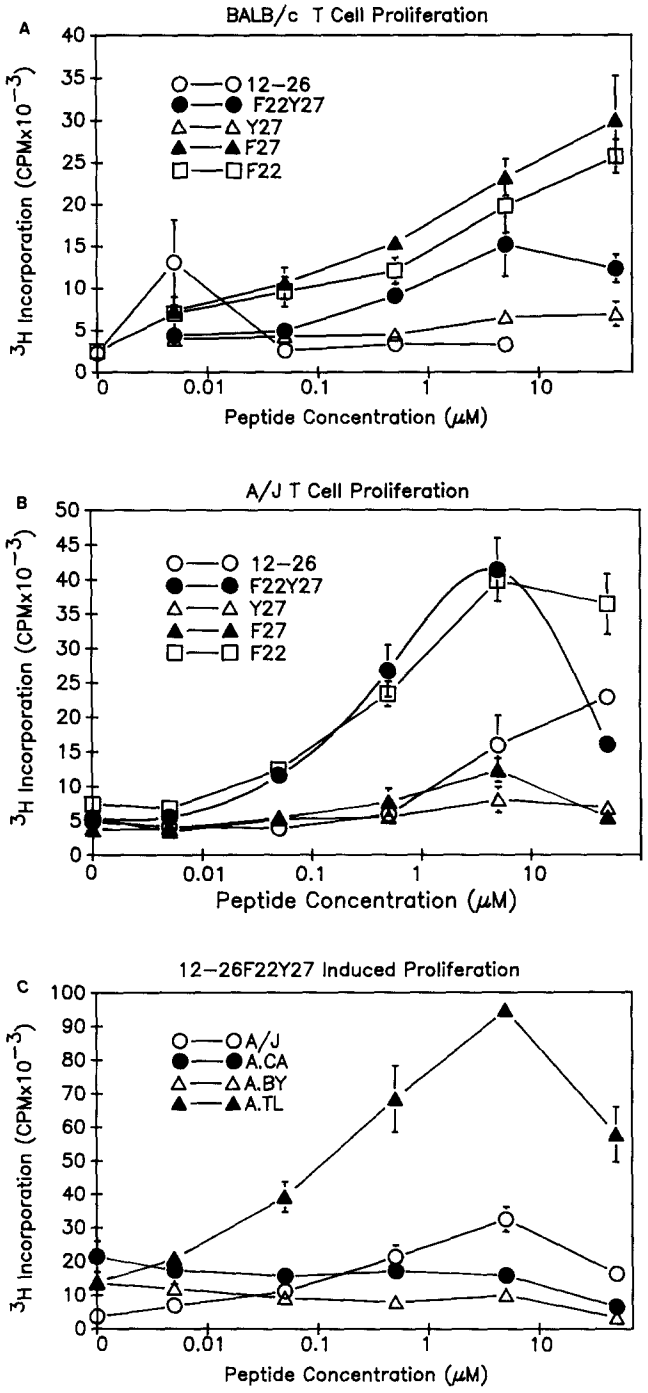

Figure 1. Bulk T cell responses of lymph node cells from inbred and MHC congenic mice to the peptide 12-26 and 12-26 sequence variants. Bulk LN proliferation assays were conducted as described in Materials and Methods. The data presented represent an average of data from three independent cultures per point. Error bars indicating SDs are shown. The proliferative responses obtained with A/J, BALB/c, and A strain MHC congenic mice are shown in separate panels. In the case of A/J anti-12-26 and anti-12-26F22Y27 responses, supernatants were harvested 2 d after initiation of culture and assayed for the presence of IL-2 using the CTLL.2 indicator line. In both cases, the amount of IL-2 in the cultures correlated with the level of proliferation as measured by [^{3}H]thymidine incorporation.

parison of the data presented in Tables 1 and 2 and Fig. 1 reveals that the magnitude of the in vitro T cell response to a given peptide is not indicative of its ability to induce hypersensitivity in vivo.

Analysis of Antibody Responses to the 12-26-based Peptides. The antibody isotype involved in immediate-type hypersensitivity (allergy, anaphylaxis) in humans is IgE (30). In mice, either IgE or IgG1 may be involved (30). Table 3 shows that peptide-specific antibody of both these isotypes can be detected in primary immune sera of many mice that are hypersensitized. Further, neither the IgG1 or IgE isotypes appear at significant levels until ~2 wk after primary immunization (data not shown). These kinetics correlate with the kinetics of sensitization of the mice; a boosting injection of peptide before 3 wk after primary immunization fails to elicit a hypersensitivity reaction.

Peptide-specific IgE cannot always be detected in primary sera of mice that are hypersensitized, and when such antibody is detected, its estimated levels are low (50–500 ng/ml). If IgE is indeed the isotype responsible for the establishment of the hypersensitivity observed, our inability to detect peptide-specific antibody of this isotypic class in some of the hypersensitized mice may be due to several factors. First, peptide-specific IgE is not detected in mice that will become hypersensitized until ~14 d after primary immunization and declines thereafter (data not shown). Since primary bleeds on most mice were done at 21 d, the "peak" serum IgE level may well have been missed in many cases. Second, the sensitivity of the passive cutaneous anaphylaxis IgE assay, in our hands, is ~50 ng/ml of antigen-specific IgE. In some cases, this may not be adequate to allow detection of peptide-specific IgE levels that are sufficient to sensitize mice. Finally, the IgE relevant to the state of hypersensitivity is presumably that bound to mast cells and basophils and not that found in the circulation.

Nevertheless, a correlation between the presence of peptide-specific serum IgE and the state of hypersensitivity is equivocal. Further, IgG1 is the predominant peptide-specific isotype present in all mice that are hypersensitized. Since this isotype has also been implicated in the development of immediate hypersensitivity in mice, it may be of central importance in establishing the peptide-induced hypersensitivity described here. However, since peptide-specific IgG1 is sometimes observed in the absence of hypersensitivity (see below), this conclusion remains tentative.

The Role of Interleukin 4 in the Development of Peptide-induced Hypersensitivity. It has been previously shown that the CD4 T cell–derived cytokine IL-4 is necessary for the production of IgE during polyclonal B cell responses in vitro (31) and in vivo (32). This cytokine also promotes the expression of the IgG1 isotype in vitro (33–35). The isotypic profile of antipeptide antibody in sera of hypersensitized mice suggests that IL-4 is involved in the regulation of isotype switching within the B cell population responding to peptide immunization. Table 4 shows that treatment of A/J mice with the anti-IL-4 mAb 11B11 (26) during the primary anti-12-26F22Y27 response dramatically reduced the number of mice

Table 3. *Peptide-specific Antibody Responses of Mice to 12-26 and Several 12-26 Sequence Variants*

Peptide	Strain	Hypersensitive Response	Peptide-specific antibody					
					Percent of total isotypes			
			κ	IgE	IgM	IgG1	IgG2	IgG3
12-26	C.AL-20	Yes	120	+				
	BALB/c	Yes	58	–	100	91		0
	A/J	No	35	–				
12-26F22	C.AL-20	Yes	1,300	+	3	97		0
	CAF1	Yes	810	–				
	A/J	No	45	–				
12-26F27	CAF1	Yes	1,600	+	2	97	1	0
	A/J	Yes	280	+				
12-26Y27	C.AL-20	No	140	–				
	A/J	No	37	–				
	BALB/c	No	30	–				
12-26F22Y27	CAF1	Yes	1,100		16	84	0	0
	C.AL-20	Yes	820	+				
	A/J	Yes	580	+	3	93	4	0
	A.TBR16	Yes	250	–	25	75	0	0
	A.TL	Yes	230	–	44	56		
	A.TBR1	Yes	180	–	28	72	0	0
	A.CA/Sn	No	92	–	21	3	73	3
	B10.A	Yes	80	–	10	90		0
	A.BY/ Sn	No	37	–				
	A.TBR2	No	20	–	100	0		
	A.TH	No	<20	–				
	A/J (anti-CD4)	No	<20	–				
	BALB/c *nu/nu*	No	22					

Different strains of mice were immunized, bled at 21 d, and challenged with the indicated peptides, as described in Materials and Methods. When assays of the following types were not performed, this is indicated by a blank. The "κ" values represent the serum dilution factor necessary to reduce binding of serum antipeptide antibodies bearing κ light chains to a peptide-BSA conjugate to one-half saturation (22). The "IgE" values represent whether or not a PCA reaction was obtained with a 1:10 dilution of serum or greater (none of the samples gave reproducible PCA reactions at a >1:100 dilution, which corresponded to ~50 ng/ml of an antiarsonate IgE mAb that was used as a positive control). The heavy chain isotype values represent the relative serum dilution factors necessary to reduce the binding of a given heavy chain isotype to a peptide-BSA conjugate to one-half saturation (22). These values are presented as the fraction of specific isotype measured as compared to a sum of the dilution factors giving half saturation obtained for all the measured isotypes. In cases where only a subset of the isotypes were measured, the isotypes that were not measured are indicated by a blank. Also shown are the results of assays done on mice treated with the anti-CD4 mAb GK1.5 and BALB/c nude mice that had been immunized with 12-26F22Y27 (see text).

showing symptoms of hypersensitivity upon secondary challenge. Moreover, antipeptide antibody of the IgE isotype could not be detected in the sera of such mice, while the average levels of both κ light chain–bearing and IgG1 anti-peptide antibody were only slightly diminished as comparable to controls. In addition, in several of the 11B11-treated mice that did not show symptoms of hypersensitivity, the levels of peptide-specific IgG1 were higher than in the untreated controls (data not shown). These data are in accord with those of others showing that in vivo IgE responses are much more susceptible than are IgG1 responses to inhibition by anti-IL4 (32), and also indicate that IgE may be of primary importance in the establishment of peptide-induced hypersensitivity in this case.

The IL-4 dependence of hypersensitivity and the lack of correlation between a peptide's ability to induce a T cell

Table 4. *Effect of Administration of an Anti-IL-4 mAb on the Development of Hypersensitivity*

Condition	Mice displaying symptoms of hypersensitivity	Peptide-specific antibody						
				Percent of total isotypes				
		κ	IgE	IgM	IgG3	IgG1	IgG2a	IgG2b
12-26F22Y27	7/8	160	+	0	1	85	4	10
12-26F22Y27 and α-IL-4 (11B11)	2/8	98	–	0	1	63	12	24

Two groups of eight A/J mice each were primed with the 12-26F22Y27 peptide in CFA as described in Materials and Methods. One group was injected intraperitoneally at 2–3-d intervals with 0.5 ml of ascites fluid containing ~500 μg of the anti-IL-4 mAb 11B11 (26), starting 1 wk before immunization. The 11B11 injections were continued until 1 mo after immunization. 3 wk after immunization, blood samples were taken from the mice, samples from the same group were pooled, and assayed for the presence of peptide-specific IgE and other isotypes as described in Materials and Methods. 1 mo after immunizaiton, both groups of mice were challenged with 12-26F22Y27 in PBS, and symptoms of hypersensitivity evaluated as described in the text and in the legend to Table 2. The levels of total antipeptide antibody bearing κ light chain and of various isotypes were determined and are indicated as described in Materials and Methods.

proliferative response and its ability to induce hypersensitivity suggested that IL-4 expressing peptide-specific CD4 T cells might be absent from mice that did not develop hypersensitivity but present in those that did. To investigate this issue, T cell hybridomas were generated from A/J mice either 7 or 30 d after immunization with 12-26F22Y27, which induces hypersensitivity in this strain, and 30 d after immunization with 12-26, which fails to induce hypersensitivity in A/J mice. The resulting hybridomas were then challenged in vitro with the immunizing peptide using a B lymphoma APC line, and IL-2 and IL-4 production was assayed. Table 5 shows that >50% of the hybridomas elicited at 30 d with 12-26F22Y27 produce both IL-2 and IL-4 upon challenge, while only two of the hybridomas elicited at 30 d after immunization with 12-26 produce IL-4 in addition to IL-2. The production of IL-2 by these hybridomas may not be an accurate indication of the status of IL-2 production by their T cell precursors, since the fusion partner used for construction of these cell lines (BW5147) secretes IL-2 upon activation (36).

Interestingly, all of the 12-26F22Y27-induced hybridomas isolated at day 7 produce IL-2 in response to antigen challenge, but none produce significant amounts of IL-4. The absence of IL-4-producing CD4 T cells early in immune responses has been observed by others (37–39), and indicates that the IL-4 phenotype must be developed within the responding CD4 T population during the course of the primary response. This early absence also correlates with the inability to induce a hypersensitive response 1 wk after immunization of A/J mice with 12-26F22Y27.

Lack of B Cell Epitopes Does Not Explain the Differential Induction of Hypersensitivity by Different Peptides. As shown in Table 3, most peptide-strain combinations that do not display hypersensitivity are also characterized by low peptide-specific serum antibody levels. Comparison of these data with those in Fig. 1 demonstrates that this lack of induction of hypersensitivity and poor humoral responsiveness does not correlate with inability to induce a T cell proliferative response. However, this inability could result from the B cell compartment of a particular strain of mouse being inefficient at recognizing a given peptide. The data presented in Tables 2 and 3 are rather uninformative in this regard, since the strain A congenics that do not develop hypersensitivity in response to immunization with 12-26F22Y27 express class II alleles that have previously been shown not to be good restricting elements for the 12-26 region of cI repressor (27). Therefore, to further investigate this issue, BALB/c and its MHC congenic strain counterpart BALB.K were used. Since both these

Table 5. *IL-2 and IL-4 Production by T Cell Hybridomas Made at 7 or 30 d after Immunization of A/J Mice with 12-26 or 12-26F22Y27*

Condition	Total no. of antigen-specific hybridomas	IL-2 Producers	IL-2 and IL-4 Producers
12-26F22Y27, day 7	37	37	0
12-26F22Y27, day 30	72	35	37
12-26, day 30	21	19	2

Experiments were conducted as described in Materials and Methods. Assignments to the "IL-2 producers" or "IL-2 and IL-4 producers" categories were based on two independent assays. In the case of the hybridomas that produced both IL-2 and IL-4 upon peptide stimulation, dose-response titrations revealed that in all cases the dose of peptide required to give half-maximal IL-2 production was always less than that required to give half-maximal IL-4 production. Interestingly, however, the ratio of these values varied over a 100-fold range.

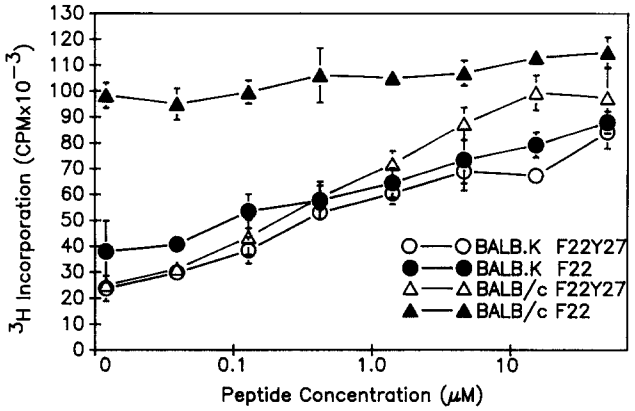

Figure 2. Proliferative responses to the peptides 12-26F22 and 12-26F22Y27 of LN cells from BALB/c and BALB.K mice. LN T cell proliferation experiments were conducted as described in Materials and Methods using lymph nodes from at least four mice per condition. Each data point represents an average of results from four independent cultures, and error bars indicating SDs are shown.

strains of mice express class II alleles (I-A^d or I-E^k) previously shown to be restricting elements for the 12-26 region (18, 29), we anticipated that a T cell immune response would be induced by 12-26-based peptides in both strains of mice. Indeed, Fig. 2 shows that 12-26F22 and 12-26F22Y27 both elicited LN T cell proliferative responses in both strains. In addition, 12-26F22Y27 induced high levels of serum antibody dominated by IgG1, as well as hypersensitivity in both these strains (Table 6). In marked contrast, 12-26F22 induced only very low levels of serum antibody in both strains, and hypersensitivity only in BALB/c. Since these two strains differ only at the MHC, and, thus, should express identical antibody V region repertoires, the differential induction of serum antibody responses and hypersensitivity by 12-26F22 cannot be due to differences in the ability of the B cell compartment of these two strains to recognize this peptide. These data further support the conclusion that the ability of a given peptide to induce a T cell proliferative response does not correlate with its ability to induce antibody production and hypersensitivity.

Discussion

Peptides representing an immunodominant CD4 T cell epitope of the bacteriophage λ cI repressor that differ subtly in primary structure induce immune responses in mice that differ both quantitatively and qualitatively. Peptides that induce hypersensitivity elicit IgG1, and many times IgE responses as well, but sometimes fail to elicit T cell proliferative responses. Peptides that do not induce hypersensitivity usually do not elicit good antibody responses but can induce vigorous T cell proliferative responses. Further, such differences can also be observed when a particular peptide is used to immunize mice that differ only in MHC haplotype.

Our results raise concerns about the general use of small synthetic peptides as human vaccines and therapeutic antagonists of activation of certain subsets of T cells. Recent experiments have shown that ability to induce hypersensitivity is not idiosyncratic to 12-26 and its amino acid variants, a peptide representing amino acids 130–142 of the influenza hemagglutinin, can also elicit hypersensitivity in certain strains of mice when injected in CFA. Our finding that CFA is a better adjuvant than Alum for the induction of hypersensitivity by these peptides appears to contrast with the work of others using high molecular weight protein antigens. Such antigens induce immunity when administered in CFA and elicit persistent IgE responses only when administered at low doses, usually on Alum (40–42). We are unaware of any previous reports showing that levels of protein-Alum–induced serum IgE correlate with the ability to induce systemic anaphylaxis in mice, however. In addition, the peptide-induced hypersensitivity described here is usually correlated with high levels of serum antipeptide IgG1 and low, transient levels of

Table 6. *Peptide-specific Antibody and Hypersensitive Responses in BALB/c and BALB.K Mice*

Strain	Peptide	Hypersensitive Response	Peptide-specific antibody					
				Percent of total isotypes				
			κ	IgM	IgG1	IgG2a	IgG2b	IgG3
BALB/c	12-26F22Y27	+ + + +	1,000	0	91	5	4	0
BALB.K	12-26F22Y27	+ + + +	610	0	93	4	3	0
BALB/c	12-26F22	+ + +	36	0	79	11	10	0
BALB.K	12-26F22	–	33	0	71	16	13	0

At least four mice for each condition were immunized, bled, boosted, and evaluated for symptoms of systemic anaphylaxis as described in the legends to Tables 1 and 2. Peptide-specific antibodies of the κ and heavy chain isotypic classes were measured in pooled serum samples as described in the legend to Table 3 and Materials and Methods. The extremely low levels of serum antibody in mice immunized with 12-26F22 were not due to this response being dominated by λ light chain bearing antibodies, since reactivity of the anti-12-26F22 antibody present in sera from such mice is >10-fold lower than the anti-12-26F22Y27 antisera with the anti-IgG1 reagent.

antipeptide IgE. Paradoxically, this type of isotypic profile is often observed in response to protein antigens administered in CFA (43, 44). Determining the immunological basis for the qualitative difference in outcome of immune responses to high molecular weight protein antigens versus the hypersensitivity-inducing peptides described here clearly requires further investigation.

It is tempting to interpret our results within the context of those of others suggesting that at least two subsets of mouse CD4 T cells exist: Th1 and Th2 (45). Cell lines representing these subsets differ both with respect to their requirements for activation (46, 47), and the cytokines they produce (45). Th2 cells produce IL-4, -5, and -6 upon activation and serve as efficient B cell helpers (48, 49). Th1 cells produce IL-2, IFN-γ, and TNF, and appear to be responsible for the induction of cell-mediated immune responses such as delayed-type hypersensitivity (50, 51). Our data are consistent with the notion that whether a T cell proliferative (Th1?) or B helper response (Th2?) is developed by the CD4 T cell population depends on both the nature of the antigen and the MHC haplotype. Other investigators have noted a dichotomy between the T cell proliferative responses and the response that generates T cells that can help B cells to secrete antigen-specific antibody in vitro based on peptide antigen or MHC differences (52–54). Further, Bottomly and colleagues (55) have shown that the CD4 T cell response to type IV collagen is of a Th1 type in A.SW mice but of a Th2 type in A.BY mice (55). The dichotomy we have observed between 12-26-based peptide-induced T cell proliferation and hypersensitivity, antibody, and IL-4 production may be reflective of a far greater diversity of CD4 T cell functionally induced by different peptide–class II combinations.

Three general models, which are not mutually exclusive, can be proposed to account for these results: (*a*) CD4 T cell subsets committed to different cytokine phenotypes express different antigen receptor repertoires; (*b*) The density of the class II–peptide ligand for the TCR on APCs either determines the cytokine phenotype to which an activated CD4 T cell will differentiate, or determines which cytokine-committed CD4 T subset will be activated; and (*c*) distinct APC are involved in the generation and/or presentation of different immunogenic peptides to CD4 T cells, and by virtue of their production of different "costimulatory" factors for CD4 T cells, either determine the cytokine phenotype to which an activated CD4 T cell will differentiate, or determine which phenotype-committed CD4 T cell subset is activated. Support for models *b* and *c* can be garnered from the literature. High levels of signalling through the TCR or CD3 complex inhibit the proliferation of Th1 cells but not Th2 cells (56–58), these cell types appear to use different "second messenger" pathways for TCR signal transduction (47, 59), and the production of particular cytokines by CD4 T cells depends on the nature of the stimulatory signal used (60, 61), supporting model *b*. Th2 cells require IL-1 for initiation of autocrine proliferation, while Th1 cells do not (46, 62), and B cells serve as better APC than adherent cells for Th2 cells, but not for Th1 cells (39, 47), supporting model 3.

In the case of the peptide-induced immune responses described here, the first model would require that the antigen receptor repertoires of different CD4 T cell subsets be capable of distinguishing peptides that differed by as little as a single amino acid residue (e.g., 12-26F22 vs. 12-26F22Y27). While this notion remains to be tested, it seems unlikely given the general diversity of specificities resident in the mouse α/β TCR repertoire (63). The third model either requires a mechanism that targets subtly different peptides to distinct APC, or a mechanism that results in differential stability of a particular peptide in distinct APC. Since current knowledge of antigen trafficking and how the antigen processing machinery present in different types of APC might differ is limited, further speculation regarding this model must await further data. The second model is consistent with the present understanding of peptide-MHC interaction in that changes in the agretypic interactions of a peptide and class II MHC antigen could be translated into differences in the density of their complex on the surface of the APC. Such differences might be transduced into different levels of T cell cytokine production if the cytokine genes required different levels of TCR complex–derived "second messengers" for their expression. An evaluation of the validity of these models in the case of immune responses elicited by 12-26-based peptides is clearly required. This will necessitate characterization of the receptor repertoires, cytokine phenotypes, and in vitro APC preferences of CD4 T cells elicited by 12-26 peptides that either do or do not elicit humoral responses and hypersensitivity, as well as measurement of the affinities of MHC class II molecules for such peptides.

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