

Peripheral T-cell tolerance induced in naive and primed mice by subcutaneous injection of peptides from the major cat allergen Fel d I

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ABSTRACT T cells control the majority of antigen-specific immune responses. Therefore, influencing the activation of the T-cell response in order to modify immune responsiveness is an obvious therapeutic goal. We have used a mouse model of response to Fel d I, the major cat protein allergen in humans, to explore the ability of peptides derived from Fel d I to inhibit T-cell-dependent immune responses to the peptides themselves and to larger polypeptides. T cells from B6CBAF₁ mice respond to the Fel d I peptide IPC-2 after challenge with IPC-2. However, subcutaneous tolerization with IPC-2 prevents this response as measured by production of interleukins 2 and 4 and interferon γ . Fel d I immunization of B6D2F₁ mice results in T-cell responses primarily to one peptide derived from Fel d I. Injecting this peptide in soluble form inhibits T-cell activation (as measured by interleukin 2 production) and antibody production in Fel d I-primed animals when they are subsequently challenged with peptide in adjuvant. Most of the cat-allergic human T-cell response to Fel d I is specific for two peptides on one of its two chains. Immunization of B6CBAF₁ mice with recombinant Fel d I chain 1 results in T-cell responses to the same peptides. Subcutaneous administration of these two peptides, which contain some, but not all, of the T-cell epitopes from Fel d I chain I, decreases the T-cell response to the entire recombinant Fel d I chain 1. The ability to tolerize T-cell responses with subcutaneous injections suggests a practical approach to treating human diseases with peptides containing T-cell epitopes.

The ability to specifically control T-cell responses would allow the control of many human diseases such as allergy and autoimmunity. Control of antigen-specific T-cell activation is therefore a focus of research in molecular and cellular immunology. T cells express receptors that recognize the complex of an antigenic peptide and a major histocompatibility complex (MHC)-encoded protein (1–3). Experiments with human and mouse T-cell clones have demonstrated that the interaction of the T-cell receptor and the peptide/MHC protein complex leads to either activation or inactivation of the cell's ability to respond to subsequent peptide/MHC challenges (4–6). Whether the T-cell receptor engagement activates or inhibits the T cell's ability to subsequently respond to the peptide/MHC protein complex may be determined by the presence or absence of costimulatory signals delivered to the T cell by the antigen-presenting cell (7, 8). An example is the signal generated by CD28 binding of B7 (9, 10). Costimulatory events of this type appear to be an obligate requirement for T-cell activation even when the T-cell receptor is bound to an appropriate MHC protein and peptide. Interestingly, if there is no second signal, then the antigen-specific T cells will become tolerized, entering into a state of

anergy. Additionally, *in vivo* tolerance to antigen challenge has been shown using soluble antigens (11, 12) or immunogenic peptides administered in such a way as to preclude the second signal (13, 14). Peripheral T-cell tolerance to endogenous antigens has also been demonstrated in MHC gene transgenic mice *in vivo* (ref. 15; reviewed in refs. 16 and 17).

We report results from an animal model of immune tolerance that may mimic certain aspects of antigen-specific T-cell tolerance in allergic humans undergoing allergen immunotherapy. Allergic individuals respond to exposure to environmental antigens with local and systemic inflammatory responses that include the production of IgE (18). It has been shown that Fel d I is the major allergenic protein produced by cats (*Felis domesticus*) (19) and the two chains of this protein have been cloned, sequenced, and expressed (20, 21). Mapping of the human T-cell epitopes for Fel d I has demonstrated that the majority of the T-cell response is specific for a broad area of the protein contained within two peptides (R. Garman and J.L.G., unpublished data). We have used these two peptides in modeling the immune response to Fel d I in several mouse strains.

MATERIALS AND METHODS

Protein and Peptides. An overlapping set of peptides derived from the sequence of Fel d I chain 1 (20) was synthesized using conventional Merrifield solid-phase *t*-Boc or Fmoc chemistry. The peptides were purified by reverse-phase HPLC and examined by amino acid analysis. The amino acid residues of each peptide are shown in Table 2. The lyophilized peptides were resuspended in phosphate-buffered saline (PBS) and sterilized by γ irradiation [10,000 rad (1 rad = 0.01 Gy)] or by passage through a 0.2- μ m filter.

Native Fel d I protein was purified from an extract of house dust as described by Chapman *et al.* (22). Briefly, house dust (from vacuum containers used in homes with multiple cats) was extracted with PBS, lyophilized, and redissolved in water. The extract was applied to a column coupled with anti-Fel d I monoclonal antibody (hybridomas 6F9 and 1G9 were provided by M. Chapman, University of Virginia). The Fel d I was eluted from the column with 100 mM glycine (pH 2.5) and was neutralized. Recombinant Fel d I chain 1 was expressed and purified as described by Rogers *et al.* (21). Fel d I chain 1 was $\approx$ 96% pure and contains the amino acids glycine and serine from a thrombin cleavage recognition site at the N terminus followed by the complete 70-amino acid Fel d I chain 1 sequence (19).

Mice. Age-matched B6D2F₁ ($H-2^b \times d$) and B6CBAF₁ ($H-2^b \times a$) (The Jackson Laboratory) 6- to 10-week-old female mice were used in each experiment.

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Abbreviations: MHC, major histocompatibility complex; IL, interleukin; IFN, interferon; CFA, complete Freund's adjuvant.

Injections. For induction of tolerance, mice were injected at a dorsal s.c. site between the forelimbs with 300 μ g of soluble protein or peptide in 0.2 ml of Dulbecco's PBS on each of 2 days, 10 and 5 days before a challenge injection. Antigen challenge was 100 μ g of either peptide or the chain 1 protein in 0.2 ml of complete Freund's adjuvant (CFA), injected s.c. at four sites at the base of the tail and in both thighs. To prime mice before tolerization, 100 μ g of Fel d I protein in 0.2 ml of incomplete Freund's adjuvant was injected intraperitoneally.

Culture Conditions. The inguinal, paraaortic, and popliteal lymph nodes and the spleens were removed from the animals 10 days after antigen challenge. The cells from these organs were suspended by being forced through a stainless steel mesh with a glass pestal. The cells were washed twice in RPMI 1640 medium with 1% fetal calf serum (FCS) before culture. All cells were cultured at 37°C in 5% CO₂ in RPMI 1640 with 10% FCS (no. F4884, Sigma), 100 units of penicillin G per ml, 10 μ g of streptomycin per ml, 10 mM glutamine, and 0.05 mM 2-mercaptoethanol. Cells were cultured in triplicate, 0.2 ml per well at 4×10^6 cells per ml, in 96-well plates. Supernatants were removed from the wells after 24 hr to test for the production of interleukin 2 (IL-2) and were frozen to kill any transferred cells. Separate triplicate wells were incubated for 48 and 72 hr, respectively, for the measurement of IL-4 and interferon γ (IFN- γ) and the supernatants were kept at 4°C for several days to kill transferred cells.

To measure IL-2, supernatants were tested after thawing for the ability to support the growth of an IL-2-dependent line, CTLL-2. CTLL-2 (a gift from D. Raulet, University of California, Berkeley) was maintained by passage in 10% supernatant of Con A-stimulated (5 μ g/ml) rat spleen cells. This subline of CTLL-2 is dependent almost exclusively on IL-2 for growth and survival, proliferating only weakly to high doses of recombinant IL-4 (unpublished observation). CTLL-2 cells (5×10^3 per well) in logarithmic-phase growth were washed twice and added in 50 μ l of complete medium to 50 μ l of warmed supernatants. After a 24-hr incubation, the CTLL-2 cells were pulsed with [³H]thymidine (1 μ Ci per well in 50 μ l of medium; 1 Ci = 37 GBq) for 4–6 hr and the plates were harvested with a 96-well harvester (Tomtec, Orange, CT) and counted on a Betaplate scintillation counter (Pharmacia). All experimental cpm values were within the linear portion of the standard IL-2 titration curves run in parallel with each assay. The background values for IL-2 production by lymph node cells in cultures with medium alone were all <500 cpm. The plateau of thymidine incorporation at high levels of IL-2 standard in these assays was between 50,000 and 80,000 cpm.

Peptide-specific IL-4 production was measured by the ability of the supernatants to sustain cell division of CT.4S cells (gift of W. Paul, National Institute of Allergy and Infectious Diseases), a cell line that grows in response to IL-4. Incorporation of [³H]thymidine was assessed as described above.

ELISA. For the IgG assay, Fel d I or IPC-2 was coated onto Polysorp (Nunc) or Immulon II (Dynatech) 96-well plates, respectively, by incubation of 50 μ l per well of 2 μ g of Fel d I per ml or 20 μ g of IPC-2 peptide per ml in PBS overnight at 4°C. Subsequent incubations were for 1 hr at 37°C. The wells were washed with PBS containing 0.05% Tween 20 and blocked with 0.5% gelatin in PBS. Sera were diluted in PBS as noted in the individual assay and were incubated in triplicate on the washed plates. After incubation and washing, the bound mouse antibody was detected by incubation with biotinylated goat anti-mouse IgG (Southern Biotechnology Associates). Streptavidin conjugated to horseradish peroxidase (Southern Biotechnology Associates) was added to detect antigen-bound biotinylated antibody complexes. 3,3',5,5'-Tetramethylbenzidine peroxidase sub-

strate (Kirkegaard & Perry Laboratories) was used according to directions supplied, and resulting OD (450 nm) values were determined using an ELISA reader (Bio-Tek model 310, Winooski, VT). The serum titer is defined as the last dilution⁻¹ at which the OD value was twice the background of preimmune serum binding to the same antigen-coated plates.

The ELISA used to detect antigen-specific binding of IgG1, IgG2a, IgG2b, IgG3, and IgM isotypes was similar to the IgG assay with the exception that biotinylated anti-immunoglobulin was used to detect the bound isotype. These assays employed biotinylated goat anti-IgG1 (no. 1070-08), anti-IgG2a (no. 1080-08), anti-IgG2b (no. 1090-08), anti-IgG3 (no. 1100-08), and anti-IgM (no. 1020-08), all from Southern Biotechnology Associates. Antigen-bound IgE was detected similarly except using biotinylated EM95 (a gift of F. Finkelman, Uniformed Services, University of the Health Sciences, Bethesda, MD), a rat monoclonal antibody specific for mouse IgE (23). Biotinylated goat anti-rat immunoglobulin (Kirkegaard & Perry Laboratories) was used as an additional signal amplification step in the IgE ELISA.

Murine IFN- γ in primary culture supernatants was detected by ELISA by binding to Nunc Polysorp wells coated with monoclonal anti-murine IFN- γ (purified from culture supernatants of the XMG1.2 hybridoma, gift of T. Mosmann, University of Alberta, Edmonton) at 50 μ g/ml in PBS (2.5 μ g per well) overnight at 4°C. Subsequent ELISA steps and incubations were as described for Fel d I or IPC-2-specific IgG with the exceptions that (i) the wells were blocked with 1% bovine serum albumin (fraction V, Sigma) in PBS for 2 hr at 37°C or overnight at 4°C, (ii) a standard of recombinant mouse IFN- γ (Genzyme) and supernatant dilutions were made in medium containing 5–10% serum, and (iii) antibody-bound IFN- γ was detected by incubation with biotinylated monoclonal hamster anti-murine IFN- γ (unlabeled, Genzyme).

RESULTS

s.c. Peptide Inhibits the Response to Subsequent Peptide Challenge. To assess the ability of s.c. administered peptide to affect a T-cell response, two groups of five B6CBAF₁ mice were treated with s.c. injections of either IPC-2 in PBS or PBS alone. B6CBAF₁ mice were chosen because of their strong T-cell response to IPC-2. The mice were then challenged with IPC-2 in CFA. The peptide-specific T-cell response was measured by IPC-2-specific IL-2, IL-4, and IFN- γ production 10 days after challenge. As shown in Table 1, IPC-2-specific production of all of the examined cytokines by lymph node or spleen cells was decreased in cells from the peptide-tolerized animals. Note that in each of the cytokine assays there were similar low backgrounds using cells from both groups of animals in cultures with no IPC-2. This experiment demonstrates that s.c. peptide injection can induce peptide-specific T-cell tolerance as shown by the inability of the cells to specifically produce cytokines. Interestingly, the production of IL-4 was decreased in the cultures from peptide-tolerized animals. In addition, antigen-specific T-cell proliferation was decreased in the peptide-tolerized animals (data not shown).

Peptide Administered s.c. Tolerizes the Peptide-Specific T Cells in Primed Mice. We were interested in examining whether peptide-induced tolerance could be demonstrated in animals already exposed to the antigen under conditions where antigen-primed T- and B-cell responses were present. This situation may better reflect clinical conditions in which an ongoing antigen-specific immune response is involved in a disease state.

The effects of s.c. peptide administration on animals primed with Fel d I were examined. One hundred eight days after priming B6D2F₁ mice with Fel d I in incomplete

Table 1. Tolerization by s.c. injection with a peptide decreases the T-cell response to that peptide

s.c. tolerization*	Cells†	<i>In vitro</i> IPC-2, $\mu\text{g/ml}$	IL-2, $\text{cpm} \times 10^{-3}$	IL-4, $\text{cpm} \times 10^{-3}$	IFN- γ , OD ₄₅₀
PBS	Lymph node	150	49.86 $\pm$ 6.46	2.82 $\pm$ 0.29	1.20 $\pm$ 0.02
		0	2.61 $\pm$ 0.98	0.56 $\pm$ 0.03	0.06 $\pm$ 0.01
	Spleen	150	31.62 $\pm$ 4.54	13.31 $\pm$ 2.96	1.14 $\pm$ 0.01
		0	2.72 $\pm$ 0.85	1.17 $\pm$ 0.14	0.06 $\pm$ 0.01
IPC-2	Lymph node	150	6.82 $\pm$ 0.64	1.04 $\pm$ 0.08	0.26 $\pm$ 0.11
		0	2.60 $\pm$ 0.74	0.39 $\pm$ 0.03	0.08 $\pm$ 0.01
	Spleen	150	4.53 $\pm$ 0.32	1.09 $\pm$ 0.20	0.09 $\pm$ 0.01
		0	1.60 $\pm$ 0.70	1.09 $\pm$ 0.11	0.07 $\pm$ 0.01

Each value in columns 4–6 represents the arithmetic mean of values from triplicate cultures $\pm$ SEM.

*Five naive B6CBAF₁ mice were tolerized by s.c. administering either IPC-2 peptide in PBS or PBS alone. They were then challenged with IPC-2.

†Draining lymph nodes and spleens were removed and pooled and cells were cultured with IPC-2 as shown. Supernatants were analyzed for cytokine production.

Freund's adjuvant, Fel d I-specific antibodies were detected, with titers $>13,500$ in sera from all of these mice. B6D2F₁ mice were chosen because of their strong response to IPC-2. Nine of the primed mice with the highest anti-Fel d I antibody responses were divided into three equal groups, matched for Fel d I-specific antibody response. There were no detectable antibodies specific for IPC-2 in any of these sera at this time (data not shown).

Spleen cells from one group were used to determine whether there was a primed T-cell response to IPC-2. The *in vitro* stimulation of spleen cells from each of the three animals demonstrated Fel d I-specific IL-2 production (Fig. 1A). All three animals also showed IPC-2-specific IL-2 production (Fig. 1B) and indicate that in this mouse strain the T-cell response to IPC-2 can account for most of the T-cell response to Fel d I. There is little or no T-cell response to either Fel d I or IPC-2 in unprimed mice. Therefore, 108 days after a priming with Fel d I, IPC-2-specific T cells and antibodies specific for Fel d I could be detected.

The remaining two groups of three Fel d I-primed animals were treated s.c. on day 109 either with IPC-2 in PBS or with PBS alone. The animals were challenged on day 119 with IPC-2 in CFA. On day 129, the draining lymph nodes were tested for specific T-cell responses to IPC-2. The peptide-specific IL-2 production by lymph node cells was significantly decreased in the peptide-tolerized mice compared to the control mice (see Fig. 2A). This experiment demonstrates that in animals with a preexisting immune response to peptide, s.c. peptide can decrease (tolerize) the ability of T cells

from these animals to respond to subsequent antigenic challenge with the peptide.

s.c. Tolerization with IPC-2 Can Diminish the Subsequent Peptide-Specific Antibody Response in Animals Primed with Fel d I. To further assess the effect of peptide tolerization on a primed helper T-cell population, we assayed the antibody response to IPC-2 following challenge with IPC-2/CFA. The

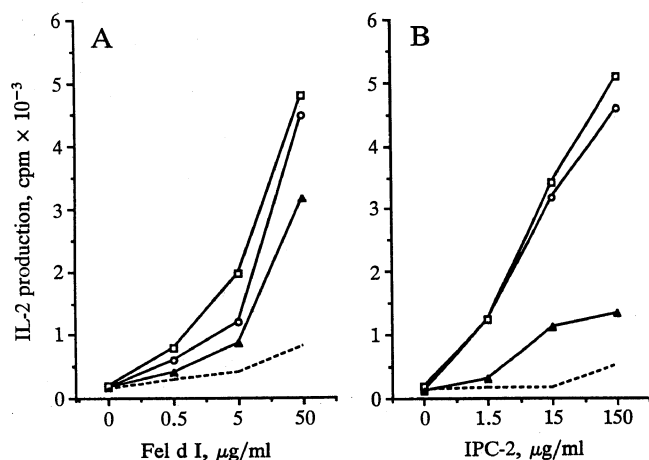


FIG. 1. T cells from Fel d I-primed mice produce IL-2 in response to IPC-2 or Fel d I. Spleen cells from three B6D2F₁ mice injected with Fel d I in incomplete Freund's adjuvant 108 days previously (three symbols with solid lines) or spleen cells from an unprimed mouse (dotted line) were cultured with Fel d I (A) or IPC-2 (B) in order to measure specific IL-2 production.

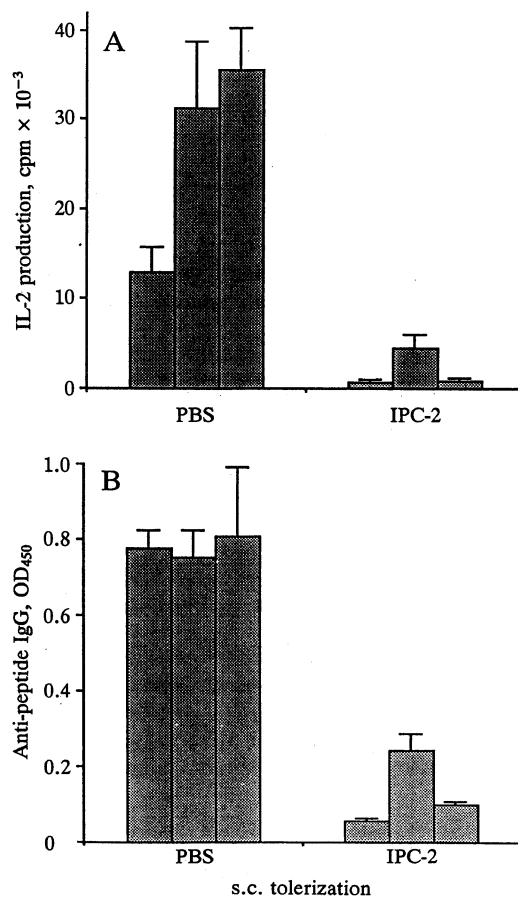


FIG. 2. Primed animals can be tolerized for specific T- and B-cell responses by s.c. injection of peptide. B6D2F₁ mice were immunized with Fel d I. After 4 months, animals were treated by s.c. injection of IPC-2 in PBS or of PBS alone. After the animals were challenged with IPC-2, the lymph node cells were assayed for IPC-2-specific IL-2 production (A). A serum sample taken at the time of sacrifice was assayed at a 1:900 dilution for antibody binding to IPC-2 (B). The background for the binding of normal mouse serum to IPC-2-coated plates or for the binding of the immune serum to uncoated, blocked plates was <0.1 O.D. unit. Each bar represents the arithmetic mean $\pm$ SEM for triplicate cultures or ELISA wells for an individual animal.

tolerized and control groups of animals described above were bled on day 129, 10 days after the IPC-2/CFA challenge. The sera were assayed for IgG antibody binding specifically to IPC-2, shown in Fig. 2B. In marked contrast to the control group, the IPC-2-tolerized animals made little antibody specific for IPC-2 in the 10 days after IPC-2/CFA challenge. The data from the other serum dilutions (from 1:300 to 1:1800) also showed significantly decreased IPC-2-specific responses. These results suggest that the s.c. peptide tolerization of primed animals decreases peptide-specific T-cell helper activity.

To determine whether the tolerization affected the isotype distribution of the IPC-2-specific antibody, the sera were assayed for the presence of IPC-2-specific IgG, IgE, and IgM isotypes. No binding above background was detected in assays for IPC-2-specific IgG2a, IgG3, or IgE (data not shown). However, IgG1, IgG2b, and IgM titers from the three IPC-2 tolerized animals were all significantly decreased relative to the PBS-injected controls (data not shown). Thus, s.c. exposure to peptide decreased the peptide-specific antibody response in primed animals without changing its isotype distribution.

s.c. peptide-induced tolerance resulted in a decrease in the production of IPC-2-specific antibody, demonstrating that peptide-specific T-cell help is affected by s.c. IPC-2 injection. Tolerization with IPC-2 had no effect on antibody specific for Fel d I in the 20 days after the first s.c. peptide injection, as assayed by specific binding to Fel d I (data not shown).

s.c. Injection of Immunodominant Peptides Decreases the T-Cell Response to Recombinant Fel d I Chain 1 Protein. To test the effects of tolerization with constituent peptides on the response to a larger polypeptide, we screened B6CBAF₁ mice for their T-cell responses to peptides following immunization with recombinant Fel d I chain 1. We used recombinant chain 1 Fel d I as an available source of standardized antigen. The pooled lymph node cells were cultured with recombinant chain 1 or with each of several overlapping peptides. The antigen-specific IL-2 production is shown in Table 2. The T-cell response to this polypeptide includes responses to several of its constituent peptides. There is a strong peptide-specific T-cell response to sequences within two peptides, IPC-1 and IPC-2. There is also a response to the region of chain 1 represented by peptides Fel 23 and Fel 21-2.

Since IPC-1 and IPC-2 contain some, but not all, of the T-cell epitopes specific for chain 1 in B6CBAF₁ mice, we determined the effect of tolerization with these two peptides on the T-cell response to recombinant chain 1, compared to tolerization with chain 1 itself. In addition, these two peptides account for the majority of human T-cell reactivity to Fel d I in T cells from cat-allergic patients (R. Garman and J.L.G.,

Table 2. B6CBAF₁ T-cell IL-2 production after recombinant Fel d I chain 1 challenge

Antigen*	Fel d I chain 1 amino acid residues	IL-2 production, [†] cpm × 10 ⁻³
Medium alone	—	5.1
Fel 1-2	1-17	4.5
Fel 2	9-25	9.5
IPC-1	7-33	15.1
IPC-2	29-55	18.5
Fel 15	44-60	6.6
Fel 23	51-66	15.3
Fel 21-2	56-69	12.7
Chain 1	1-70	23.1

*A pool of lymph node cells from 10 recombinant Fel d I chain 1-primed animals was cultured with 150 μ g of each overlapping peptide per ml. The specific IL-2 production for each of the peptides is shown.

[†]Each value represents the arithmetic mean from triplicate cultures.

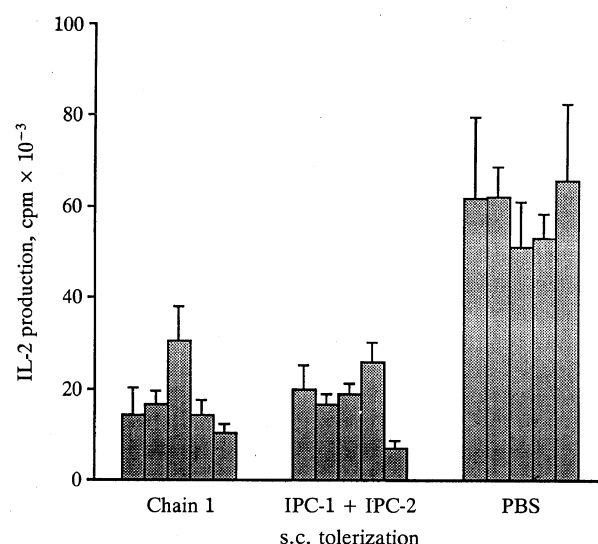


FIG. 3. Tolerization with peptides containing immunodominant epitopes can tolerate for challenge with protein. Four groups of five B6CBAF₁ mice were treated with two s.c. injections of either 150 μ g of recombinant Fel d I chain 1, 150 μ g each of a combination of peptides, or PBS alone. Each mouse was challenged with recombinant Fel d I chain 1. The lymph node cells were cultured with 150 μ g of recombinant Fel d I chain 1 per ml or with medium alone. Each bar represents the arithmetic mean $\pm$ SEM for values derived from triplicate cultures for an individual mouse.

unpublished observations). Three groups of mice were treated by s.c. injection of either 150 μ g of recombinant Fel d I chain 1, 150 μ g of IPC-1 and IPC-2, or PBS alone. All of these s.c. injections were in 0.3 ml total volume. The mice were then challenged with recombinant Fel d I chain 1 in CFA. The lymph node cells were cultured with recombinant Fel d I chain 1. Tolerization with peptides or Fel d I chain I led to decreased chain 1-specific IL-2 production in these mice (see Fig. 3). The tolerization produced by IPC-1 and IPC-2 was as effective as tolerization with chain 1. The peptide-specific T-cell responses were also assayed. All of the peptides tolerized the T-cell response to themselves when assayed by culture with each peptide *in vitro* and did not tolerate the T-cell response to other nonoverlapping peptides (data not shown). Treatment of the mice with IPC-1 and IPC-2 did not increase or decrease the T-cell responses to other T-cell epitope-containing peptides (data not shown).

These experiments demonstrate that s.c. injection with peptides derived from Fel d I chain 1 decreases the T-cell response to challenge with chain 1. It was not necessary to tolerate all of the T cells specific for chain 1 in order to significantly decrease the chain 1-specific T-cell response, demonstrating that tolerance to a protein can be elicited using peptides containing some, but not all, of the T-cell epitopes.

DISCUSSION

Effective intervention in allergic and other immune response-mediated diseases requires altering an established or ongoing immune response. We have shown that s.c. administration of peptide decreases the T-cell response to subsequent challenge with the same peptide, whether the animals are naive or specifically preimmunized. For such tolerization with peptides to be clinically useful, tolerization must be effective for environmental exposure to whole protein. Our experiments show that tolerization with two peptides from Fel d I chain I decreases the response to challenge with the larger protein subunit. These data suggest the possibility of peptide-induced T-cell tolerance as a therapeutic approach in immune-mediated diseases. The ability to tolerate with s.c.

injection of peptide rather than intravenous or intraperitoneal injection (e.g., refs. 13 and 14) would allow this to be a practical therapeutic approach.

T-cell responses that are not modulated by thymic education may be regulated in the periphery by several mechanisms, including clonal anergy (reviewed in refs. 16 and 17), clonal elimination (24), and antigen-specific suppression of the T-cell response (reviewed in ref. 25). s.c. injection of peptide without adjuvant is used here to elicit antigen-specific anergy by presenting antigen to T cells in the absence of costimulatory signals. In such a setting, the antigen-presenting cell may not provide a second signal needed to activate the T cells (26). We cannot rule out clonal elimination of peptide-specific T cells as a mechanism for induction of tolerance in the above experiments until we recover anergic T cells. Using *in vitro* mixing experiments we have detected no suppressive effector function in cells from tolerized animals (unpublished observation). Based on the data available, the most likely explanation for our results is the induction of antigen-specific peripheral T-cell anergy.

Most *in vitro* models of anergy have shown tolerization of Th₁-type cells (27). However, recent reports also have shown *in vitro* tolerization of both Th₀- and Th₂-type T-cell clones (28, 29). The extent of the decrease in peptide-specific IL-2, IL-4, and IFN- γ production that we have shown suggests that peptide-specific T cells of Th₁ and Th₂ type are affected by our tolerization procedure. In addition, the observation that peptide-specific production of antibody is inhibited in the 10 days after peptide/CFA challenge of Fel d I-primed animals suggests that peptide-specific T cells with Th₂ or Th₀ functions are affected by this tolerization. The demonstration here that s.c. treatment with peptide can decrease the peptide-specific antibody production without changing the antibody isotype distribution suggests that peptide-induced T-cell tolerance decreases T-helper function without altering the balance of Th₁ vs. Th₂ helper function for antibody production. The constant titers of Fel d I-specific antibody during tolerization and challenge may reflect the normal half-life of antibody in these mice.

Several recent papers (30, 31) have shown evidence of selective tolerization of Th₁ cells after intraperitoneal injection of soluble whole proteins. Although those reports differ from our results, the different routes of antigen administration and the use of large doses of whole proteins may account for the different outcomes of the tolerization. Another recent paper (32) suggests that intraperitoneal injection of soluble protein inhibits T-helper cell activity as read out by IgG1 production by memory B cells. We postulate that s.c. tolerization with peptides decreases all T-cell effector functions specific for that peptide through inhibiting T-cell clonal expansion. In support of this, we find that peptide-induced tolerance inhibits peptide-specific proliferation as well as cytokine production (data not shown).

The experiments reported here show that peptides containing some of the T-cell epitopes in a larger polypeptide can tolerate T cells for subsequent challenge with that larger polypeptide. As shown in Fig. 3, the degree of T-cell tolerization achieved with the two peptides IPC-1 and IPC-2 was indistinguishable from that achieved with tolerization with chain 1. This points to the utility of controlling an immune response with a limited number of T-cell epitope-containing peptides. s.c. injection of two peptides from the major allergen of cat can decrease the T-cell response in mice to a single chain of the Fel d I protein. This suggests that in humans with a similar distribution of T-cell specificity, most of the T-cell response to this protein might be tolerized by using two peptides. The tolerization of the allergen-specific

T-cell response could lead to clinical relief of allergic symptoms.

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