~90,000, ~20,000, ~2,000, and 60 cDNA clones. A single clone (pKI27) was finally obtained.
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23. We thank M. Kuno, T. Tsujimoto, T. Takahashi, and Y. Okada for discussions and T. Miyata for computer analysis. Partly supported by research grants from the Ministry of Education, Science, and Culture of Japan, the Institute of Physical and Chemical Research, and the Science and Technology Agency of Japan.

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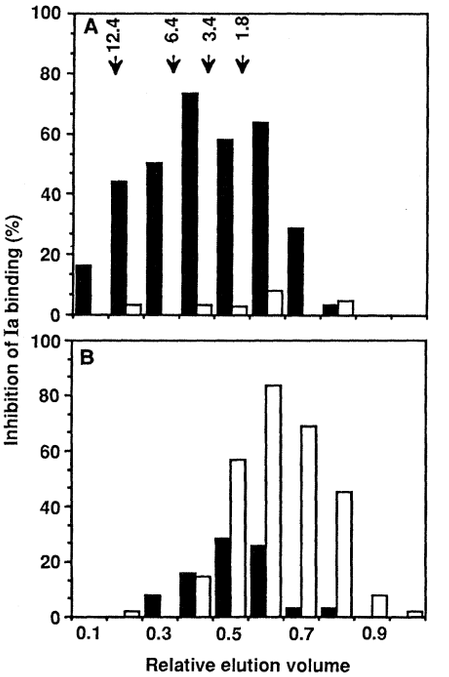

Fig. 1. One of two experiments showing the Ia binding capacity of material obtained from acid-treated Ia. Acid-treated I-A^d (**A**) and I-E^d (**B**) were gel-filtered and assayed for I-A^d (filled bars) and I-E^d (open bars) binding activity. I-A^d–derived material bound exclusively to I-A^d and not I-E^d. I-E^d–derived material bound predominantly but not exclusively to I-E^d. Ia molecules were affinity-purified as described (*3*) and either treated with acid or left untreated for peptide binding assays. Briefly, 200 μg of I-A^d or I-E^d in phosphate-buffered saline was exposed to 2.5*M* acetic acid for 20 min at 37°C, and then separated according to size by Sephadex G-50 chromatography (buffer: 0.04*M* ammonium acetate and 0.02*M* acetic acid). Fractions (10 ml) were collected and lyophilized four times. Each fraction was taken up in 50 μl of buffered saline solution, and 10 μl was tested for its capacity to inhibit the binding to Ia of a radiolabeled peptide [Ova (Y 323-339) for I-A^d and lambda repressor C1 (Y 12-26)], which binds to I-E^d (*5*). The relative elution volume was calculated as $(V_e - V_o)/V_t - V_o)$, where V_e is elution volume, V_o is void volume, and V_t is total volume. The positions of markers of known molecular weights are indicated in (A).

Autologous Peptides Constitutively Occupy the Antigen Binding Site on Ia

SØREN BUUS, ALESSANDRO SETTE, SONIA M. COLON, HOWARD M. GREY*

Low molecular weight material associated with affinity-purified class II major histocompatibility complex (MHC) molecules of mouse (Ia) had the expected properties of peptides bound to the antigen binding site of Ia. Thus, the low molecular weight material derived from the I-A^d isotype was efficient in inhibiting the binding of ^{125}I-labeled I-A^d–specific peptide to I-A^d, but did not significantly inhibit the binding of an I-E^d–specific peptide to I-E^d; the reciprocal isotype-specific inhibition was demonstrated with low molecular weight material derived from I-E^d. The inhibitory material was predominantly peptide in nature, as shown by its susceptibility to protease digestion. It was heterogeneous as measured by gel filtration (mean molecular weight ~3000), and when characterized by high-performance liquid chromatography, it eluted over a wide concentration of solvent. Such self peptide–MHC complexes may have broad significance in the biology of T cell responses, including generation of the T cell repertoire, the specificity of mixed lymphocyte responses, and the immune surveillance of self and nonself antigens in peripheral lymphoid tissues.

IN GENERAL, T CELLS OF THE HELPER subset recognize complexes of altered—that is, denatured or fragmented—protein antigen and MHC Ia molecules (*1–3*). Ia molecules act as receptors (*4*) with a single binding site for "processed" antigen (*5, 6*). The permissive specificity of Ia—probably through the broad recognition of peptide "motifs"—allows each Ia to bind many different peptides (*5, 7*). Ia does not distinguish between peptides representing self and nonself (*8*).

Circumstantial evidence suggests that Ia may bind peptides derived from endogenous, as well as exogenous proteins. First, Babbitt *et al.* (*8*) demonstrated that I-A^k was capable of binding the autologous mouse lysozyme peptide 46-61 and the homologous xenogenic hen egg lysozyme peptide equally well. Second, Björkman *et al.* (*9, 10*) examined the x-ray crystallographic structure of a human MHC class I molecule, HLA-A2, and found evidence of electron-dense material occupying the putative antigen binding site. Finally, we found that only 5% to 10% of affinity-purified Ia molecules are capable of binding peptide and have speculated that this could be caused by the presence of self peptides constitutively present in the binding site of Ia (*11*).

To evaluate whether peptides constitutively bound to Ia would likely remain bound during the affinity purification of Ia, we exposed radiolabeled peptide-Ia complexes to the same conditions as used in the affinity purification procedure. Our purification procedure involved a high *p*H (*p*H 10 to 10.5) elution step at 4°C lasting 5 to 10 min. At least for the interaction of ovalbumin (Ova) peptide 323-339 and I-A^d, only a minor fraction (approximately 2%) of peptide-Ia complexes was dissociated under these conditions. These results are consistent with the possibility that affinity-purified Ia might have a low peptide binding capacity because its antigen binding site is already occupied to a large extent with self peptides.

Department of Medicine, National Jewish Center for Immunology and Respiratory Medicine, Denver, CO 80206.

*To whom correspondence should be addressed at Cytel, 12520 High Bluff Drive, Suite 250, San Diego, CA 92130.

In contrast, exposing complexes of Ova(323-339) and I-A^d to *p*H 2.5 for 5 minutes resulted in complete dissociation of the complexes.

With this information, we decided to use an acid dissociation procedure to determine whether, from affinity-purified Ia, we could elute low molecular weight material that had the expected Ia binding properties of peptides derived from the antigen binding site. Acid-treated Ia was fractionated by gel filtration to separate Ia from low molecular weight material, and the fractions were tested for their capacity to bind I-A^d or I-E^d as measured by their capacity to inhibit the known binding of a radiolabeled peptide to Ia. Low molecular weight Ia binding activity was eluted from both I-A^d and I-E^d. Figure 1 shows one of two such experiments. Although not absolutely specific for the Ia from which it was derived, the binding activity was highly selective, especially for the I-A^d–derived material, which bound very well to I-A^d and insignificantly to I-E^d. The I-E^d–derived material bound preferentially to I-E^d, but some I-A^d binding was also observed. This lack of absolute specificity was in keeping with our experience with synthetic peptides, since we have found that about 8% of peptides capable of binding to one isotype (I-A^d or I-E^d) could also bind to the other isotype. As judged by the elution profile, the mean molecular weights (M_r) of the I-A^d– and I-E^d–derived material were approximately 3500 and 2000, respectively. The inhibition profiles were distributed in single peaks, which were much broader than the peak distribution of a single model peptide of similar size. This is compatible with the existence of considerable size heterogeneity of the material with Ia binding activity. Although there was too little peptide present in the low molecular material eluted from the G-50 columns to allow detection of protein or peptide by either a colorimetric assay or by silver staining of SDS-urea-polyacrylamide gels, we were able to detect peptides by radioiodinating a pool of the low molecular weight material. SDS-urea-polyacrylamide gel electrophoresis (PAGE) and autoradiography of this labeled pool gave a smear in the range of 2000 to 12,000 M_r, with no discrete bands being observed.

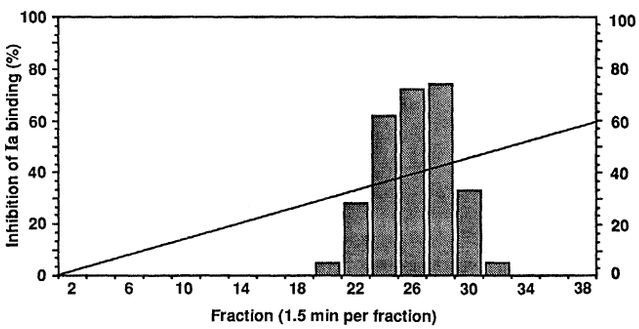

Fig. 2. One of two experiments showing HPLC separation of low molecular weight material obtained from I-A^d. A broad peak of I-A^d binding activity which eluted at 23% to 43% acetonitrile was obtained. Ia (200 μg) was acid-treated and separated by G-50 gel chromatography. The low molecular weight material was pooled and subjected to reversed-phase HPLC on RP-300 (Brownlee) C_8 column with a linear gradient of acetonitrile in 0.1% trifluoracetic acid. Fractions (1.5 ml) were obtained, lyophilized twice, and tested for their I-A^d inhibitory activity as described for Fig. 1.

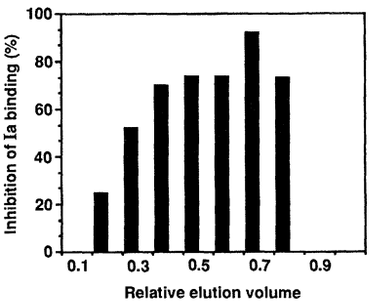

Fig. 3. Acid-treated I-A^k obtained from tumor AKTB-1b grown in vivo was prepared and gel-filtered as described for Fig. 1. The capacity of the fractions to inhibit the binding of ^{125}I-labeled hen egg lysozyme (HEL) peptide (Y 45–61) was measured as previously described (*5*).

To further characterize the inhibitory material eluted from Ia, we analyzed the active fractions obtained from gel filtration by reversed-phase high-performance liquid chromatography (HPLC) (Fig. 2). A broad peak of Ia binding activity was obtained that eluted over a wide concentration of acetonitrile (23% to 43%). This further substantiates the conclusion that the inhibitory activity is associated with a heterogeneous mixture of molecules.

To determine whether the substance (or substances) with inhibitory activity was peptide in nature, we tested the low molecular weight material obtained from acid-treated Ia for its sensitivity to proteolytic enzymes (Table 1). Most, but not all of the inhibitory activity was sensitive to chymotrypsin, papain, and pronase, an indication that the acid-eluted material from Ia that is capable of rebinding to Ia is predominantly peptide in nature. The failure to destroy all Ia binding activity by proteolytic enzyme treatment could be due to the presence of peptides that are particularly resistant to such treatment or, alternatively, could be due to the capacity of substances other than peptides to bind to the antigen binding site of Ia.

The removal of peptides from the antigen binding site of Ia would be expected to improve the capacity of such "stripped" Ia to bind exogenously added radiolabeled peptide. Although binding was improved up to fourfold after acid treatment, it was observed in only about half the experiments, with the other preparations either having unchanged or decreased peptide binding capacity. The reason for this inconsistency is not known, but it is likely due to the balance achieved between release of material from the binding site, which would lead to an increased binding capacity, and the denaturation of Ia caused by the *p*H 2.5 treatment,

Table 1. One of two experiments showing protease sensitivity of material derived from acid-treated Ia. I-A^d–derived low molecular weight material was tested for sensitivity to proteases. As positive controls for protease sensitivity, a tryptic digest of Ova and a purified I-A^d–binding peptide, Ova(323-336), were also tested. Ninety percent to 100% of I-A^d binding activity of the Ova peptides was destroyed by protease treatment and 65% to 80% of the I-A^d binding activity of the low molecular weight I-A^d–derived material was destroyed. Low molecular weight material obtained from 100 μg of I-A^d, 20 μg of Ova tryptic digest, and 10 μg of Ova(323-336) was incubated with 1 μg of the respective proteases for 4 hours at 37°C and then with another 1 μg of protease overnight at 37°C. Residual proteolytic activity was inhibited by boiling for 10 min and then treating with a cocktail of protease inhibitors [EDTA (3 mg/ml), 1.190 phenanthroline (0.26 mg/ml), 10 m*M* *N*-ethyl maleimide, 2 m*M* phenylmethylsulfonyl fluoride, TLCK (100 μg/ml), and TPCK (100 μg/ml)], and the inhibitory activity of the protease-treated material was assessed as for Fig. 1.

Treatment	Dose needed to obtain 50% inhibition of binding of ^{125}I-Ova(323-339) to I-A^d					
	Low molecular weight material from I-A^d		Tryptic digest of Ova		Ova(323-336)	
	μl	%	μ*M*	%	μ*M*	%
None	5	100	70	100	2	100
Chymotrypsin	14	35	730	10	255	1
Papain	13	38	>1500	0	>500	0
Pronase	25	20	>1500	0	>500	0

which would lead to a decrease in peptide binding capacity.

Although self protein would be the major source of Ia-binding peptides in vivo, we could not exclude the possibility that some of the Ia-binding peptides were derived from the fetal calf serum that was used to grow the I-A^d–expressing B cell lymphoma A20-1.11 in vitro. To address this point, we purified I-A^k from the lymphoma AKTB-1b, a tumor that is propagated in vivo. The low molecular weight I-A^k binding material that was eluted had activity similar to that obtained from the A20-1.11 cells grown in fetal calf serum (Fig. 3). Thus, we can conclude that antigen presenting cells can process self proteins of cellular or extracellular origin and that the peptide site on Ia contains such processed self peptides constitutively.

If the self peptides we have characterized are representative of naturally processed antigen, then processing would appear to generate peptides no larger than M_r 12,000, with most of the processed peptides being in the range of 2000 to 4000. The I-A^d–bound self peptides were somewhat larger than those bound to I-E^d, suggesting somewhat different structural requirements for binding to the two Ia isotypes. The fraction of Ia occupied by self peptides is unknown, but a rough estimate could be obtained from these experiments. If it is assumed that the eluted material consists of peptides with an affinity for I-A^d similar to that of the control peptide Ova(323-336), it is possible to calculate how many Ova peptide equivalents were eluted. From 3.3 nmol of I-A^d, 1.7 to 2.2 nmol equivalents (50% to 70% yield) of Ova(323-336) were obtained. This suggests that most of the Ia molecules in vivo are occupied by self peptides. Indeed, on the basis of our previous observation that only 5% to 10% of affinity-purified Ia was capable of binding exogenously added peptide, the fraction of occupied Ia may exceed 90%. This high degree of occupancy by chemically heterogeneous material suggests that Ia would appear to a T cell as very heterogeneous. As a corollary to this, T cell responses against peptides generated from foreign proteins would have to be induced by the small fraction of the total Ia available for presentation of newly processed exogenous antigen.

The existence of self peptide–Ia complexes could explain the high frequency of alloresponsive T cells since, if such T cells recognize allo-Ia plus peptide, the heterogeneity of the peptides bound to Ia would greatly expand the T cell clonotypes capable of recognizing allo-Ia. This concept is a variation of a previously proposed model for allorecognition (*12, 13*). Also, the generation of the T cell repertoire during ontogeny may be influenced by the nature of the Ia-associated self peptides in the thymus, both with respect to positive and negative selection of T cells. Finally, the binding of self peptides to MHC proteins in peripheral lymphoid tissue might provide a mechanism for immune surveillance, since neoantigens would be presented by the class I MHC of a tumor cell for recognition by killer T lymphocytes.

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A Bitter Substance Induces a Rise in Intracellular Calcium in a Subpopulation of Rat Taste Cells

MYLES H. AKABAS,* JANE DODD, QAIS AL-AWQATI

The sense of taste permits animals to discriminate between foods that are safe and those that are toxic. Because most poisonous plant alkaloids are intensely bitter, bitter taste warns animals of potentially hazardous foods. To investigate the mechanism of bitter taste transduction, a preparation of dissociated rat taste cells was developed that can be studied with techniques designed for single-cell measurements. Denatonium, a very bitter substance, caused a rise in the intracellular calcium concentration due to release from internal stores in a small subpopulation of taste cells. Thus, the transduction of bitter taste may occur via a receptor–second messenger mechanism leading to neurotransmitter release and may not involve depolarization-mediated calcium entry.

ALTHOUGH THE FUNCTION OF THE taste buds has been inferred for over 100 years, very little is known about the physiology of the taste cells or the molecular mechanisms involved in the gustatory transduction process (*1*). This lack of knowledge is due primarily to the small number of taste cells and their relative inaccessibility in situ. Recently, several patch clamp studies of isolated amphibian taste cells have identified a variety of ion channels in the taste cells, but their relation to the sensory transduction process is unclear (*2, 3*). Studies of transepithelial currents in lingual epithelium suggest that the transduction of salty, sweet, and sour tastes involves vectorial ion movements through the epithelium (*4, 5*). Bitter substances, however, induce no change in the transepithelial currents in canine lingual epithelium (*6*). To study taste transduction we developed a preparation of dissociated rat taste cells.

Taste cells from rat circumvallate papillae were identified with a monoclonal antibody (MAb) that recognizes the Lewis b blood group epitope. This epitope is expressed selectively on the taste cells (Fig. 1A) and on the overlying squamous cells (*7*). After dissociation of the epithelium by a combination of enzymatic digestion and mechanical agitation (legend to Fig. 1), the MAb labeled small aggregates of cells (Fig. 1B) and rare single cells. About 50% of the aggregates were labeled. Further evidence that the labeled aggregates were composed of taste cells was obtained with a Ca^{2+}–adenosine triphosphatase (Ca^{2+}-ATPase) histochemical staining procedure (*8*). Ca^{2+}-ATPase activity is associated with the taste cells in the lingual epithelium (*9*) (Fig. 1C) and, in the dissociated cell preparation, was found in the aggregates of cells (Fig. 1D, right). Thus, after dissociation, the taste buds remain as small aggregates, but the remainder

M. H. Akabas, Department of Medicine, College of Physicians and Surgeons, Columbia University, New York, NY 10032.
J. Dodd, Department of Physiology, College of Physicians and Surgeons, Columbia University, New York, NY 10032.
Q. Al-Awqati, Departments of Medicine and Physiology, College of Physicians and Surgeons, Columbia University, New York, NY 10032.

*To whom correspondence should be addressed.