HUMAN T CELL EPITOPES OF BOVINE SERUM ALUBUMIN, THE MAJOR BEEF ALLERGEN

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Background

Bovine serum albumin (BSA) is a major beef allergen. It is very recently reported by Beretta *et al.* (1) that the C terminal region of BSA may be an IgE-binding area for patients based on analyses of tryptic hydrolysates of BSA.

BSA and human serum albumins (HSA) display approximately 76% sequence homology and a repeating pattern of disulfides that is strictly conserved. The fact that a major component of serum acts as an allergen is very surprising for the following reason: it is remarkable that albumins from animals, which are very similar in sequence, structure, and function to human serum albumin, are recognized by the human immune system as allergens instead of tolerance being induced.

Allergen-specific T lymphocytes determine the quality of the subsequent antibody response by their pattern of cytokine production in response to specific activation. It is therefore necessary to analyze the immunoreactivity at the level of T cells.

Objectives

The aim of this study was to characterize T cell responses to BSA in beef-allergic patients. We hypothesized BSA-specific T cells react primarily with sequential epitopes that differ greatly between BSA and HSA, since BSA and HSA have similar tertiary structures. We found 15 regions in which the amino acid sequences of BSA and HSA differ greatly. We, therefore, synthesized peptides corresponding to such regions as candidate epitopes, and characterized the responses of T cells to these peptides.

Methods

Peptides corresponding to amino acid positions 57-65, 107-123, 123-137, 153-166, 178-191, 292-311, 336-345, 338-341, 364-382, 389-400, 431-444, 451-459, 453-457, 466-472, 497-506, 513-521, 545-552, 547-550, and 573-581 of BSA (*see* figure in next page) were synthesized as candidate epitopes essentially according to the solid phase method using a peptide synthesizer. Peripheral blood samples were obtained from four patients (Nos. 1 to 4) allergic to beef with atopic dermatitis (CAP-RAST score: meat, 4-6; milk, 3-6). Peripheral blood mononuclear cells (PBMCs) were isolated from whole blood samples of four donors, and used in the lymphocyte proliferation assays. Briefly, the PBMCs were cultured for 72 hrs in the presence of 50 microM of synthesized BSA sequence peptides. Cultures of PBMCs alone were included as negative controls. The cells were pulsed with 5-Bromo-2'-deoxyuridine (BrdU) for the final 24 hrs of culture. The BrdU incorporated by the cells was measured by ELISA. Peptides that induced 1.5 times more proliferation than the control culture were considered to show increased proliferation.

Results and discussion

Cultured PBMCs from four patients were stimulated with the synthesized peptides, and the results of the lymphocyte proliferation assays are shown in figure (*see* next page). Proliferation was seen in more than half of the patient PBMCs tested when the cells were incubated in the presence of peptide Nos. 2, 8, and 11. These three peptides were also recognized by IgE antibodies (2). Thus, all of the major T cell epitopes found in this study are concluded also to be B cell epitopes.

This is the first demonstration of the identification of human T cell epitopes of BSA. Studies of the T cell epitopes in the primary structure of other allergenic proteins have revealed that, similar to BSA, two or more major T cell epitopes usually exist in the sequence of the allergen and disperse throughout the molecule.

Specific immunotherapy protocols involve the repeated administration of T cell epitope-containing allergens over prolonged periods of time, which induces relative immunologic tolerance. However, severe adverse reactions occasionally occur during specific immunotherapy with this approach, due to the binding of the administered allergen to allergen-specific IgE antibodies bound to mast cells. This can be avoided if allergen-derived synthetic peptides are used instead of whole allergenic proteins. Among three T cell epitopes found in this study, peptide No. 11 (LSLILNRLC, aa451-459) is the shortest in length and best recognized by patients at high frequency. Since the nonapeptide LSLILNRLC might be unable to crosslink IgE antibodies and be unable to trigger the subsequent release of chemical mediators such as histamine, LSLILNRLC and/or its analog peptide(s) would be of interest for use in the treatment of beef-allergy.

In conclusion, we have identified some T cell epitope peptides in BSA, which is a major beef allergen and also famous in biochemistry. This finding would contribute greatly to the elucidation of beef allergy.

(1) Beretta B. *et al.* (2001) *Int. Arch. Allergy Immunol.*, **126**: 188-195.
(2) Tanabe S. *et al. submitted*

Peptide	Sequence	Position	Patients			
			1	2	3	4
No. 1	ESHAGCEKS	57-65				
No. 2	**DDSPDLPKLKPDPNTLC**	107-123				
No. 3	CDEFKADEKKFWGKY	123-137				
No. 4	LLYANKYNGVFQEC	153-166				
No. 5	PKIETMREKVLTSS	178-191				
No. 6	EKDAIPEDLPPLTADFAEDK	292-311				
No. 7	HPEYAVSVLL	336-345				
No. 8	**PHACYTSVFDKLKHLVDEP**	364-382				
No. 9	NCDQFEKLG	389-400				
No. 10	VGTRCCTKPESERM	431-444				
No. 11	**LSLILNRLC**	451-459				
No. 12	PVESKVT	466-472				
No. 13	PKAFDEKLFT	497-506				
No. 14	TLPDTEKQI	513-521				
No. 15	VMENFVAF	545-552				
No. 16	LVVSTQTAL	573-581				