

Clonal Analysis of the Cellular Immune Response to the House Dust Mite *Dermatophagoides farinae*

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Abstract. A panel of human CD4⁺ T cell clones specific for the house dust mite was isolated from an atopic individual with perennial rhinitis. Soluble antigen specificity was defined using proliferation assays and subsequently the antigenic determinants recognized by some of these clones were mapped using nitrocellulose immunoblots of fractionated *Dermatophagoides* spp. Both cross-reactive and species-specific T cell clones were identified and in some instances their specificity could be mapped to the serologically defined *Der f*I, *Der p*I and *Der f*II allergens. In comparison, the serum antibody response showed additional specificity for *Der f*III. The antigen recognition by six of these clones was found to be restricted by HLA-DR gene products.

Introduction

The role of T lymphocytes in the induction and regulation of allergic respiratory diseases is ill-defined. Investigation of the antigenic structures of two of the common aeroallergens, the house dust mites *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*, suggest that *Der p*I and *Der f*I (26 kilodaltons, kD) are the major allergens and an additional major allergen, *Der f*II (12.5 kD), has been described [1]. The identification of these antigens, however, was serologically determined.

In this study, therefore, a panel of human CD4⁺ T lymphocyte lines and clones specific for *Dermatophagoides* spp. was raised from an individual allergic to the house dust mite to facilitate the investigation of the cellular immune response to this aeroallergen. The soluble antigen specificity was defined and subsequently the antigenic determinants recognized by some of these clones were mapped by using the sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) immunoblot assay system for T cell antigen recognition [2]. The predominant T cell response of the clones was species-specific for *D. farinae*, the inducing antigen, and could be mapped to the serologically defined *Der f*II allergen. In contrast, analysis of the serum antibody response using Western blotting demonstrated additional specificity for

*Der f*I and *Der f*III (29 kD), and was consistent with the predominantly cross-reactive antibody response described for these allergens.

The genetic restriction of antigen recognition by the T cell clones was subsequently examined using murine monoclonal antibodies directed against human major histocompatibility complex (MHC) class II molecules to inhibit antigen-dependent proliferation. The six clones tested were all restricted by HLA-DR gene products.

Materials and Methods

Antigens

Lyophilized extracts of *D. farinae*, *D. pteronyssinus* and mixed grass pollen were generously provided by Pharmacia (Uppsala, Sweden).

Antibodies

Murine monoclonal antibodies against human MHC class II determinants L243 (anti HLA-DR), Leu-10 (anti-HLA-DQ) and B7/21 (anti-HLA-DP) were purchased from Becton Dickinson (Sunnyvale, Calif.).

Isolation of T Cell Lines and Clones

A panel of house-dust-mite-specific CD4⁺ T cell lines and clones was isolated using the limiting dilution method, as described previously [3, 4]. Before use in assay systems the lymphocytes were rested for 6-8 days after the last addition of filler cells and antigen.

Table 1. Antigen specificities of cloned T lymphocytes

	<i>D. farinae</i>	<i>D. pteronyssinus</i>	Grass pollen	No antigen
T cell lines				
DX1	+++	-	-	-
DX2	+++	±	-	-
T cell clones				
DE41	+++	-	-	-
DE9	+++	++	-	-
DD11	+++	-	-	-
DE5	+++	-	-	-
DE12	+++	-	-	-
DE26	+++	+	-	-

T lymphocytes (5×10^4 /ml) were cultured with autologous irradiated PBMC (1.25×10^5 /ml) in the presence of the inducing antigen *D. farinae*, *D. pteronyssinus* or mixed grass pollen. Proliferation as correlated with [^{3} H]-thymidine incorporation was measured at 72 h.

Preparation of Immunoblots

SDS-PAGE immunoblots of *D. farinae* and *D. pteronyssinus* were prepared as described previously [3, 5]. Briefly, antigen was boiled in the presence of SDS and 2-mercaptoethanol, separated in a 'mini-gel' apparatus and transferred to nitrocellulose by electroblotting. The sectioned immunoblots were solubilized and reprecipitated before use in proliferation assays.

Autoradiography from Immunoblots of Dermatophagoides spp.

Western blotting and autoradiography were performed from nitrocellulose-transferred SDS-PAGE immunoblots of *D. farinae* and *D. pteronyssinus* as described in detail [3].

Genetic Restriction Using T Lymphocyte Proliferation Assays

Cloned T lymphocytes were cultured with *D. farinae* in optimized autologous cultures to which murine monoclonal antibodies against human MHC class II molecules were added over a concentration range [4]. Proliferation was determined as in the legend to table 1.

Results and Discussion

The long-term T cell lines (DX1, DX2) and the T cell clones (DE41, DE9, DD11, DE5, DE12, DE26) proliferated strongly to the activating antigen *D. farinae* (table 1). DX2 gave a weak cross-reactive response to the closely related member of the same genus *D. pteronyssinus*, and DE9 and DE26 proliferated strongly to this allergen. No response was seen to the irrelevant mixed grass pollen allergen.

T lymphocytes from the line DX2 and clone

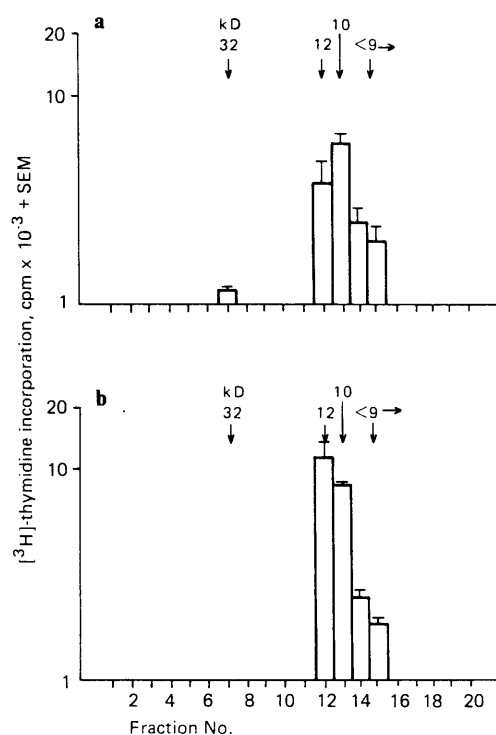


Fig. 1. Differential pattern of reactivity of the T cell line DX2 (a) and clone DD11 (b) to SDS-PAGE antigens solubilized from immunoblots of *D. farinae*. T lymphocytes (10^5 /ml) were cultured with SDS-PAGE-separated immunoblots (20 fractions) after solubilization with dimethyl sulfoxide together with irradiated PBMC (5×10^5 ml) and assayed as in the legend to table 1. (Reproduced with kind permission of Immunology [from ref. 3].)

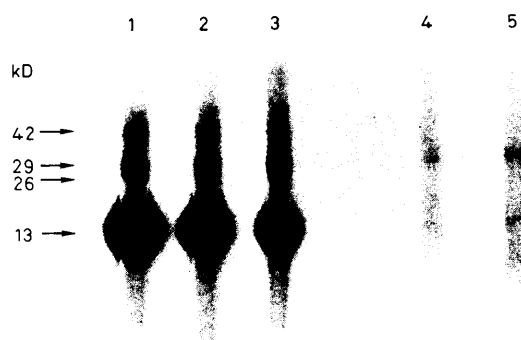


Fig. 2. Autoradiography of Western blot showing the IgE response of autologous and nonatopic allogeneic sera to *D. farinae*. *D. farinae* mite body extract was separated by SDS-PAGE and transferred to nitrocellulose. The strips were probed with sera from the mite-allergic PBMC donor (lane 1 = undiluted; lane 2 = 1:2; lane 3 = 1:5) and from a normal individual (lane 4 = 1:2; lane 5 = 1:5), and autoradiographed to detect binding of IgE.

DD11, in the presence of accessory cells, proliferated in response to *D. farinae* immunoblotted preparations (fig. 1). The predominant site of antigen recognition for the T cells from this individual was within the molecular-weight range 9–13 kD. Clone DE9, one of the two cross-reactive clones, gave a strong proliferative response in the range 24–35 kD of *D. pteronyssinus* immunoblots [5] with a similar single peak to *D. farinae* immunoblots [unpubl. data]. These determinants comigrated with the *Der p* I and *Der f* I allergens of house dust mite (26 kD) and the response pattern was confirmed using the affinity-purified preparations of these allergens kindly provided by Dr. M.D. Chapman [5]. The autoradiograph using autologous serum showed strong IgE binding at the 13-kD fraction corresponding to the major site of T cell recognition (fig. 2). Fainter bands were evident at 26, 29 and 42 kD. A similar pattern was observed to *D. pteronyssinus* immunoblots [3].

Therefore, both cross-reactive and species-specific T cell clones could be isolated but the majority of the T lymphocytes recognized species-specific determinants of *D. farinae* of molecular weight 9–13 kD. In contrast, the B cell response was consistent with the recognized cross-reactivity of the IgE response to house dust mite allergens.

Using the murine monoclonal antibodies against human MHC class II framework antigens, maximal blocking was obtained with the anti-HLA-DR antibody, suggesting restriction by immune-response genes of the HLA-DR subregion [4].

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