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USE OF MONOCLONAL ANTIBODIES FOR THE CHARACTERIZATION OF ALLERGENIC EXTRACTS

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Summary. - *The diagnosis of allergic diseases is commonly carried out by using poorly standardized preparations containing allergenic and non-allergenic components. Recently, monoclonal antibody technology has been applied to the biochemical characterization and to the purification of allergens, in order to evaluate their potential role in the standardization of diagnostic and therapeutic extracts. Three main results have been achieved: 1) the characterization of allergenic epitopes recognized by IgE and IgG from patients' sera; 2) the purification by affinity chromatography of relevant allergenic components; 3) the development of immunoassays for the quantitation of allergens in the commercially available extracts used for diagnosis and therapy.*

KEY WORDS: allergens, monoclonal antibodies, standardization.

Riassunto (Impiego di anticorpi monoclonali nella caratterizzazione di estratti allergenici). - *La diagnosi delle malattie allergiche viene comunemente effettuata impiegando preparazioni ottenute con metodi biochimici dalle varie fonti allergiche (pollini, spore, acari, epiteli, ecc.); tali preparazioni sono miscele molto eterogenee e poco caratterizzate. Recentemente la tecnologia degli anticorpi monoclonali è stata applicata alla caratterizzazione biochimica e alla purificazione di strutture allergeniche, allo scopo di valutarne le potenzialità, nella standardizzazione degli estratti allergenici. Tre approcci principali sono stati seguiti: la caratterizzazione di epitopi allergenici riconosciuti da IgE e IgG di soggetti allergici, la purificazione mediante cromatografia per affinità di allergeni rilevanti, e lo sviluppo di dosaggi immunologici per la valutazione del contenuto di allergeni presenti nei vari estratti utilizzati per la diagnosi e la terapia.*

PAROLE CHIAVE: allergeni, anticorpi monoclonali, standardizzazione.

Introduction

Allergic disease is a consequence of the hyperreactivity of the immune system towards exogenous stimuli such as pollens, mites, molds, animal epithelia, insect venoms, foods, etc. Subjects suffering from this disease produce higher amounts of a particular class of antibody, IgE, specific for these allergens, than normal individuals do. These antibodies are able to bind to the membrane of mast cells and basophils, and to induce the release of soluble mediators, once cross-linked by the allergen. These mediators are responsible of the allergic symptoms such as asthma, rhinitis, conjunctivitis, urticaria and anaphylactic reaction.

The diagnosis of allergic disease is based on the demonstration of the presence of IgE antibodies specific for the allergen. The preparations commonly employed for this purpose are poorly standardized mixtures of many allergenic and non-allergenic components and the biochemical procedures for characterizing and purifying those of interest often represent a very complicate approach.

In recent years, many studies have dealt with the isolation and chemical characterization of the various components present in the extracts from both pollens and other allergenic materials widely diffused and clinically relevant in North America and Europe.

After the monoclonal antibody (MoAb) technology became available, [1] many attempts have been made in order to successfully apply this technique to the characterization of allergenic extracts, to the purification by immunochemical procedure (immunoaffinity) of the relevant allergens, and to the development of immunoassays to measure the content of allergen(s) in an extract. The use of MoAbs presents several advantages: 1) their specificity can be defined and remains constant; 2) they can readily be produced in large quantity; 3) they can be consistently reproduced over several years.

Monoclonal antibodies in the study of allergens

Several allergenic extracts have already been studied by using MoAb technology (reviewed in [2]). In basic research, these reagents have been used to investigate the repertoire of antigenic sites on allergen molecules, as site specific probes to investigate the epitope specificity of IgE and IgG antibody responses and the intra- and inter-species allergen cross-reactions, and to study idiotypic regulation of the IgE response. MoAbs have been also employed to quantitate by two-site immunoassays the amount of relevant allergens contained in allergenic preparations used for diagnosis and immunotherapy. Moreover, major allergens from several sources have been isolated by affinity chromatography on MoAb-Sepharose. Interestingly, MoAbs have been used to localize the hysthological structure in which allergens are synthesized and eventually secreted. The studies reported above have been performed on allergens from both animal and plant origin. Chapman *et al.* produced MoAbs specific for the major allergen from *Dermatophagoides pteronyssinus* [3] (Der p I); the majority of these MoAbs were Der p I specific, isotype IgG1 and did not react with a comparable *Dermatophagoides farinae* allergen. Assessments of the specificity of these MoAbs suggested that they could be grouped into two families, each recognising an epitope on Der p I molecule. None of the MoAbs significantly inhibited human IgE binding to Der p I, although 12 out of 14 sera from mite allergic patients were able to inhibit binding by the MoAbs. The same Authors were also able to obtain cross-reactive MoAbs, recognizing common epitopes shared by Der p I and Der f I [4]. Employing both specific and cross-reactive MoAbs, two-sites radioimmunoassays (RIAs) were developed to major allergen Der p I and Der f I [5]; these assays were very sensitive (nanogram range) and highly specific, and they can measure allergen levels in mite or dust extracts without the need for purified allergen or affinity-purified antibodies. The Authors suggest that MoAbs RIAs will be useful in clinical studies, to assess patients' exposure to mite allergens and the effects of avoidance regimens.

MoAbs were also applied to the study of *Felis domesticus* major allergen Fel d I [6, 7]. Chapman *et al.* [6] were able to purify Fel d I by single-step immunoaffinity procedure employing a monoclonal antibody raised against the major feline salivary allergen. The allergen was purified from aqueous house dust extract with a high Fel d I content; this single-step procedure gave 40 to 50% recovery of pure allergen. MoAbs specific for Fel d I were also employed in the development of two-site RIAs using MoAbs directed against different epitopes on the allergen molecule [6, 7]. A similar approach was followed by Kahn and Marsh [8] in the study of *Lolium perenne* major allergen Lol p I. Several MoAbs were produced against the major allergen, one of which was found to recognise not only Lol p I allergen but also the related 27,000-30,000 Group I glycoproteins found in the pollens of nine other species of grass pollen tested. Moreover, this MoAb was used in affinity

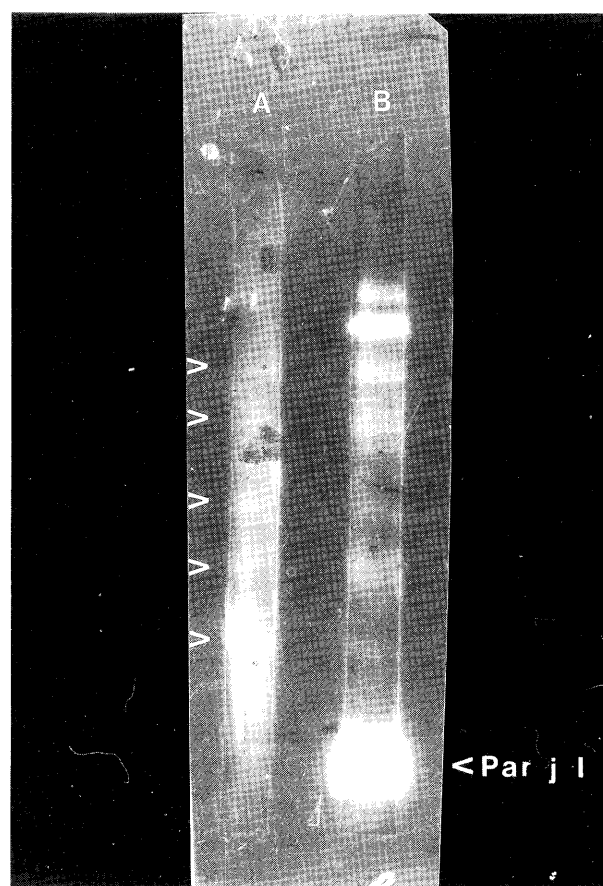


Fig. 1. - Autoradiography of the immunoblotting pattern of *P. judaica* pollen extract displayed by the monoclonal antibody 1A4/2F8 (lane A), and by a pool of individual patients' sera (lane B). White arrows indicate allergenic molecules recognized by the monoclonal antibody.

chromatography to purify Lol p I allergen and in the development of a RIA useful in the standardization of grass pollen extracts according to the Group I content. Cross-reactivity between grass pollens was also studied by Mourad *et al.* by means of MoAbs specific for Lol p I and Dac g I [9]; the results indicated that the MoAbs studied were directed against four different and non-overlapping epitopes present on both allergens, and that these MoAbs were able to inhibit to variable degree the binding of human IgE to Lol p I and Dac g I. Different MoAbs specific for other main allergens from *Lolium perenne* (Lol p IV) were used by Bose *et al.* [10] to demonstrate the presence of a common idiotope on human and murine antibodies to rye grass pollen allergen Lol p IV.

The epitope analysis by MoAbs has been also carried out on the major ragweed allergen Amb a I, which was demonstrated to bear two major human allergenic sites (A and B) [11]. The binding of IgE in pooled human serum from ragweed allergic individuals was blocked approximately 50% by a MoAb directed to site A and also approximately 50% by a MoAb directed against site B. The conclusions from these data is that site A and site B represent two very major allergenic sites in the human response to Amb a I. Other than A and B epitopes detectable in the native molecule, epitopes exposed only in the

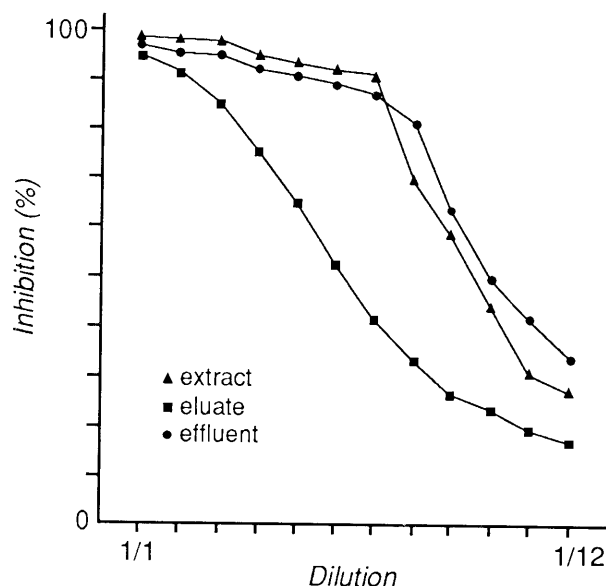


Fig. 2. - ELISA inhibition of human IgE binding to the *P. judaica* pollen extract by the whole extract (▲), and by the eluate (■) and the effluent (●) from IA4/2F8 monoclonal antibody immunoabsorbent.

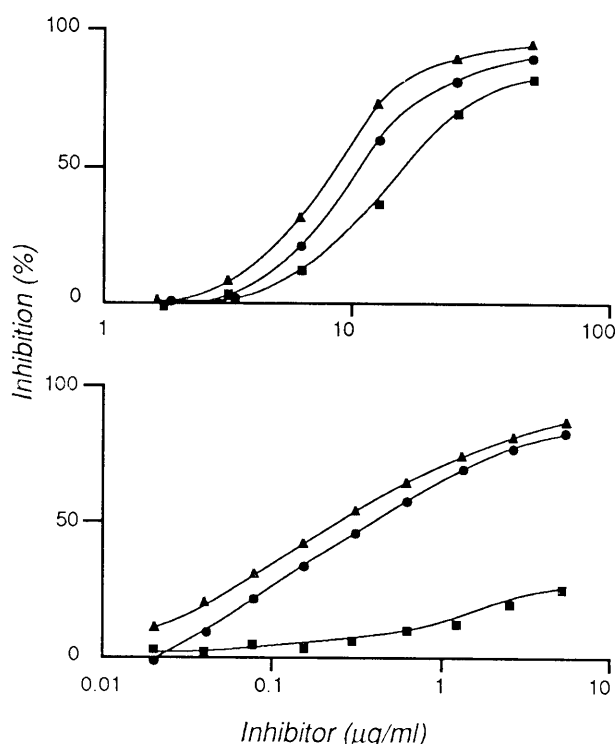


Fig. 3. - ELISA inhibition of the monoclonal antibody binding (top) and of specific rabbit antiserum (bottom) to the *P. judaica* pollen extract by the native (▲), chemically deglycosylated extract (■), and mock control (●).

denatured form of the allergen could be identified by MoAbs raised by injecting mice with reduced Amb a I [12]; none of these MoAbs was able to bind to native Amb a I. The Authors suggest that these reagents represent a powerful tool in the isolation and analysis of peptide fragments which are necessarily obtained from denatured molecules.

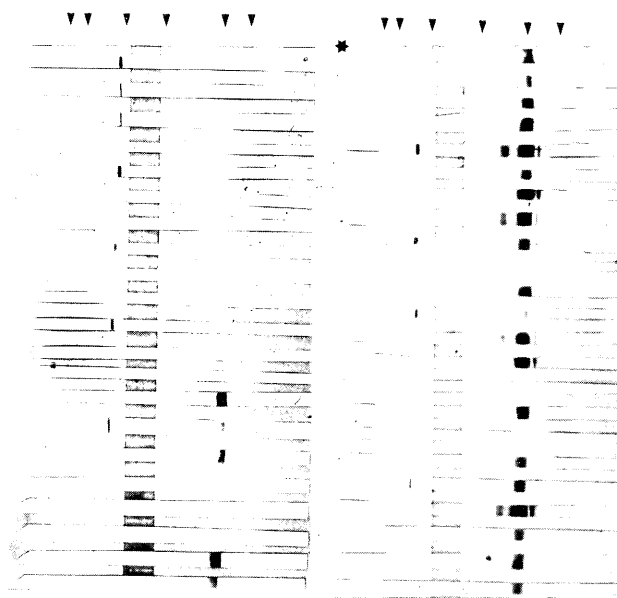


Fig. 4. - Panel of reactivity in immunoblotting against the *O. europea* pollen extract of 43 monoclonal antibodies. The lane indicated with (*) was developed with a rabbit antiserum specific for *O. europea* as control. Black arrows indicate molecular weight markers (97, 66, 43, 31, 22, 14 kDa).

Among allergenic pollens from plants relevant in the Mediterranean area, Corbi *et al.* have studied *Parietaria judaica* pollen extract by using monoclonal antibodies following two different approaches. The first one is based on an immunoaffinity procedure employing a monoclonal antibody specific for the major allergen Par j I [13]; the allergen, purified to homogeneity by this immunoabsorbent, was able to strongly inhibit most of the binding of specific IgE to the *P. judaica* pollen extract coupled to paper discs. The affinity purified allergen exhibited the same immunoelectrophoretic pattern as the native allergen. The same monoclonal antibody was able to immunoabsorb four different polypeptides from the radioiodinated pollen proteins [14], thus suggesting the presence of at least four different components sharing the same epitope. The second approach led to the development of a competitive solid-phase RIA for quantitation of the major allergen Par j I [15]; this method was successfully used for the estimation of the allergenic activity of various *P. judaica* pollen extract preparations.

In our laboratory, monoclonal antibodies against *P. judaica* pollen extract have been produced and their reactivity was characterized in ELISA and immunoblotting. Among the positive clones obtained, one, designed IA4/2F8, was of great interest, since it was able to detect in immunoblotting, even after three cloning steps, more than one component of the extract, different from the major allergen. Comparing the reactivity of this monoclonal antibody with the immunoblotting pattern displayed by a pool of individual patients sera (Fig. 1), it was possible to demonstrate that the majority of these components are allergens. This finding was further confirmed by the strong inhibiting capacity displayed in a human IgE-ELISA inhi-

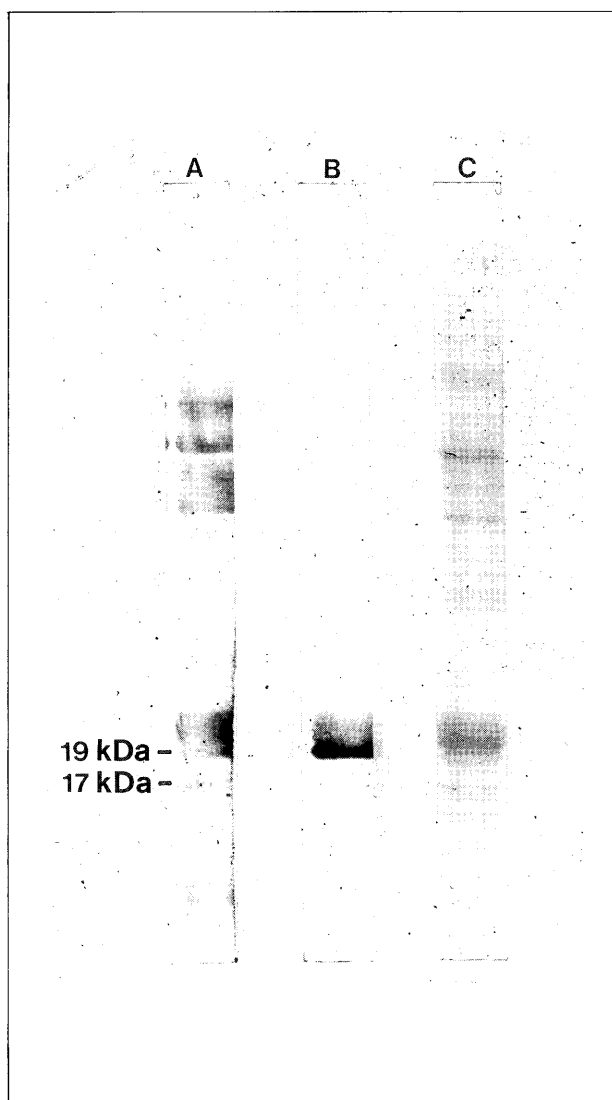


Fig. 5. - Immunoblotting of *O. europea* pollen extract, developed with polyclonal mouse serum (lane A), monoclonal antibody 2C8 (lane B), or stained with amido black (lane C).

bition experiment by the eluate from an immunoabsorbent column prepared with the same MoAb (Fig. 2). The MoAb multiple reactivity can be explained by the presence of epitopes different from and non cross-reactive with the major allergen. Our results are in agreement with those reported in the literature [14] obtained with a different MoAb, although our MoAb does not react with the major allergen in immunoblotting performed in reducing and denaturing conditions. In order to evaluate if carbohydrate moieties were responsible for this cross-reactivity, the MoAb was tested comparing native and chemically deglycosylated extract in an immunoenzymatic inhibition assay (Fig. 3, top). The results indicated that the cross-reactive epitope recognized by the MoAb is not a carbohydrate structure, since the deglycosylated extract was as effective as the native one in inhibiting the MoAb binding. On the contrary, a polyclonal rabbit antiserum specific for the *P. judaica* extract used as a control was not inhibited by the deglycosylated sample (Fig. 3, bottom).

Other than *P. judaica*, *Olea europea* pollen allergens have also been studied in our laboratory by using the MoAb technology. At present, several clones producing antibodies specific for both the 17 and 19 kDa major allergen or other minor components with higher molecular weight, as demonstrated by immunoblotting, are available (Fig. 4). A MoAb with specificity for the major allergen has been also described by Lauzurica *et al.* [16, 17]. On the basis of their data the Authors suggest that the two protein bands recognized by the MoAb share common epitopes although they possess different carbohydrate contents, as demonstrated by the different behavior on a Concanavalin A immunoabsorbent; this MoAb could inhibit the binding of human IgE from a pool of sera to the *O. europea* crude extract. In addition, we were able to produce and characterize a MoAb specific for the 19 kDa component only, designed 2C8, thus demonstrating that this molecule bears determinants not shared with the 17 kDa one (Fig. 5).

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