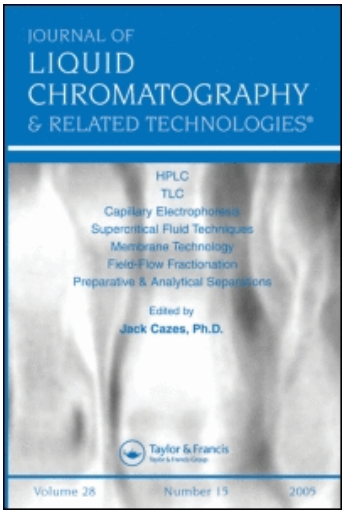

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HPLC METHOD FOR ANTIGEN-ANTIBODY INTERACTION STUDY. APPLICATION TO ANTI HIV gp120 ANTIBODY. PROPOSITION OF TREATMENT TO IMPROVE THE EFFICIENCY OF THE IMMUNE RESPONSE

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ABSTRACT

Gel filtration on HPLC columns can be a convenient means for the separation of soluble immune complexes from the mixture of human serum albumin (HSA) - monoclonal serum albumin antibody (Mab anti HSA). Owing to the rapidity of the method, the equilibrium is not modified, as it may be during the salting-out separation.
This method has been applied to the study of the interaction between HSA and a monoclonal antibody against human immunodeficiency virus envelope protein (Mab anti HIV gp120). Supported by the fact that HIV proteins share numerous epitopes with human proteins, a treatment to improve the specific immune response against this pathogen is proposed : it is based on the blocking of the epitopes of the self antigens, expressed in the thymus, which are in common with the virus. Repeated injections within the thymus, of neutralizing antibodies against the pathogen, obtained from a sufficiently distant animal species and purified by affinity chromatography, would prevent the T cells of the host recognizing these common epitopes from negative selection, and would improve the immune response. Moreover, the unfavourable effect of mutations within the virus genome could also be minimized by this treatment.

INTRODUCTION

Liquid chromatography and especially HPLC have been rarely used in immunology. This technique has been involved in a few studies, such as purification of monoclonal

antibodies on hydroxyapatite columns (1, 2), separation of immune complexes on Sephadex columns (3), or study of the topology of the epitopes at the surface of a protein (4).

We describe here the separation, by size exclusion chromatography, of human serum albumin (HSA) and one of its monoclonal antibody, from their immune complexe. We have then applied this method to the study of the interaction between HSA and a particular anti HIV gp120 antibody.

A new kind of therapy is proposed, applicable to AIDS, in order to improve the efficiency of the immune response.

MATERIALS AND METHODS

HPLC was applied with a Waters Ass. liquid chromatograph composed of a 510 pump, a U6K injector and a 996 photodiode array detector with the Millenium 2010 Chromatography Manager (Millipore).

The separations were performed on gel filtration TSK G 3000 SW columns (Beckman), in Tris sulfate buffer 0.1 M pH 8.0, at the rate of 1 ml/min.

Monoclonal antibody against HSA (Mab anti HSA A 6684) was purchased from Sigma Immunochemicals, monoclonal antibody against HIV gp120 (Mab anti gp120 1 C 1) from ICN Biomedicals and human serum albumin (HSA) from Sigma. They were repurified on TSK G 3000 SW column, in order to eliminate high molecular weight impurities or aggregates, interfering with the immune complexes.

RESULTS

a) HSA-Mab anti HSA system

HSA and monoclonal antibody anti HSA are eluted approximately at the same volume on TSK G 3000 SW with Tris sulfate buffer as eluent, and cannot be separated from each other. However they are well separated from another peak, eluting close after the void volume of the column, and visible when the two components have been incubated together before injection (Fig. 1). This peak represents the immune complex formed in the conditions used for the incubation, and the amount can be evaluated by integration, assuming in a first approximation, that HSA, its antibody and their complex have the same extinction coefficient.

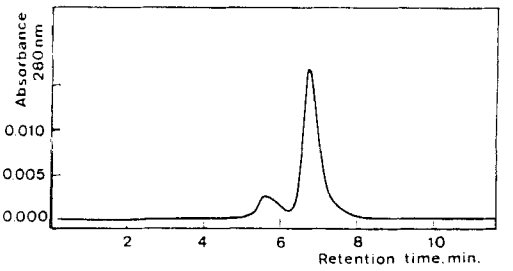

FIGURE 1 : Chromatography of HSA-Mab anti HSA mixture.
TSK G 3000 SW column, Tris sulfate 0.1M pH 8.0 buffer, rate 1ml/min,
injected HSA : 5.3 µg, injected Mab anti HSA : 9.0 µg, time of incubation : 5 min.
The first eluted peak is the immune complex.

This HPLC separation is rapid, the immune complex is eluted from the column in less than 6 min, far less than with the salting out method (5) (30 min). One must then expect that the equilibrium will be practically not disturbed during the chromatography : the proportion of the immune complex peak is actually independent of the duration of the separation as shown on Fig. 2. It has been verified that the fraction of the immune complex separated by this method does not depend on the amount of the injected mixture : Fig. 3 shows that the surface of the first peak is proportional to the injection volume. On the other hand, the equilibrium between the components of the incubated mixture is reached in less than 2 min, as can be seen on Fig. 4. The time of incubation has been fixed to 5 min for all the samples. Therefore, this method is able to give immediate quantitative results, by integration of the chromatographic peak of the immune complex. However it cannot give information about the composition of these complexes, unless the contribution of the antigen could be specifically measured.

When increasing quantities of HSA are added to a constant amount of monoclonal antibody, the immune complexe increases and reaches a plateau (Fig. 5). In the situation where HSA is constant and the added antibody increases, the same kind of curve is observed (Fig. 6). The level of the plateau is determined by the amount of the limiting component, and no decrease is observed when the other component is added in large excess. However the value of the maximum is lower than expected, on the basis of the formation of a divalent antibody-monovalent antigen complex. This discrepancy could be due to an heterogeneity of the HSA, with regard to the epitope which is recognized by the antibody.

It must be noted that the monoclonal antibody used in this study is specific of the human albumin : the level of the immune complex reached with bovine serum albumin is only 6 % of that obtained with HSA.

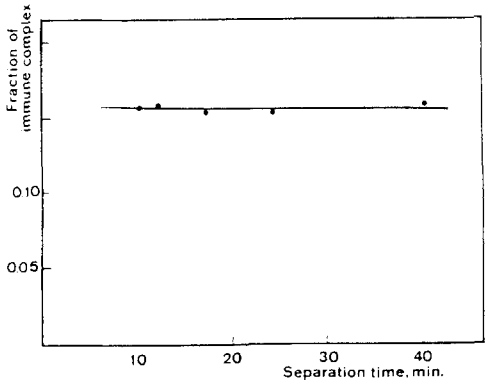

FIGURE 2 : Influence of the time of chromatography on the fraction of the separated immune complex.
Injected HSA : 10.7 µg, injected Mab anti HSA : 9.0 µg. Other conditions as in figure 1.

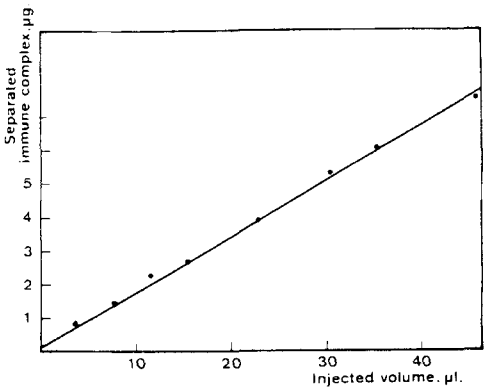

FIGURE 3 : Influence of the injected volume of the incubated mixture on the amount of the separated immune complex.
Rate 1ml/min. Same incubated mixture and other conditions as in figure 2.

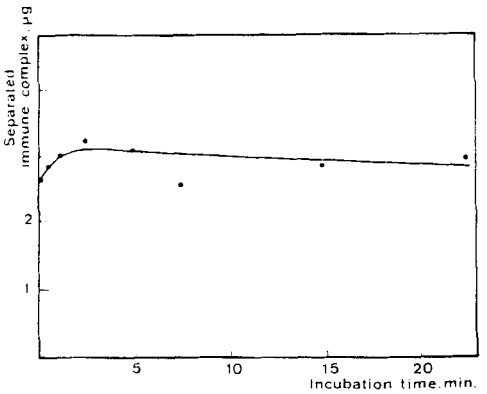

FIGURE 4 : Influence of the incubation time on the amount of the separated complex. Same conditions as in figure 2, except for incubation time.

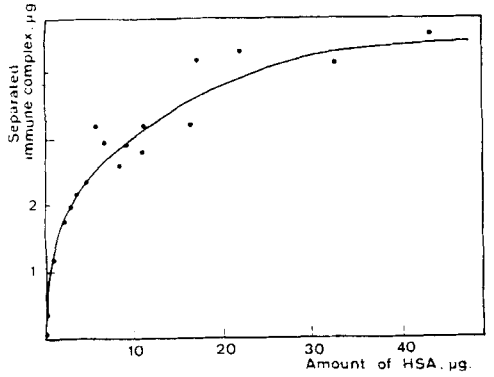

FIGURE 5 : Variation of the amount of the separated immune complex versus HSA concentration, at constant Mab anti HSA concentration.
Injected Mab anti HSA : 9.0 µg.

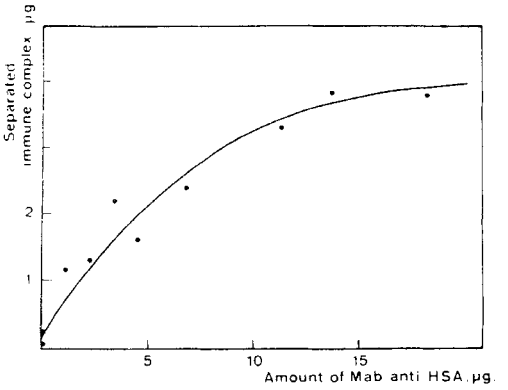

FIGURE 6 : Variation of the amount of the separated immune complex versus Mab anti HSA concentration, at constant HSA concentration.
Injected HSA : 10.7 µg.

b) HSA-Monoclonal anti HIV gp120 system

Among the different theories concerning the mechanism of T cell destruction after HIV infection, autoimmunity has been evoked by several authors. This hypothesis is supported by the presence in the sera of HIV infected individuals of antilymphocytes antibodies, antibodies to Interleukin 2, to peptides from MHC II molecules, to platelets and neutrophils and to partially sialyated form of CD 43 on normal thymocytes (6). It has also been found T lymphocytes that bind to autologous immunoglobulin (7), and cytotoxic T lymphocytes that can lyse uninfected $CD4^+$ T cells (8). These responses are thought to be due to the occurrence of regions of homology between HIV envelope proteins (gp120 and gp41) and different human proteins (MHC class I and class II proteins, Interleukin 2 etc...) (9, 10).

In this work, we have used the HPLC separation of the immune complexes, in order to determine whether other human proteins would possibly cross react with HIV envelope proteins. The system used for this purpose was HSA-Mab anti HIV gp 120. As can been seen on Fig. 7, a high molecular weight complex is separated from a HSA-Monoclonal antibody anti HIV gp120 incubated mixture. Fig. 8 shows the variation of the amount of this immune complex when the antigen increases, the other component being kept constant. Here also, the level determined by the limiting component (Mab anti HIV gp 120) is lower than expected, but the discrepancy could be attributed to heterogeneity of the antibody.

Nevertheless, from these results, one can conclude that HSA, the most abundant human serum protein, shares epitopes with the virus envelope protein gp120, like the other above mentioned proteins.

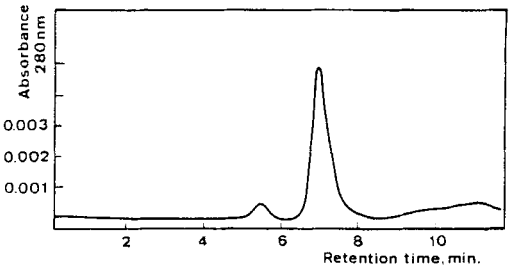

FIGURE 7 : Chromatography of HSA-Mab anti HIV gp120 mixture.
Injected HSA : 3.6 µg, injected Mab anti HIV gp120 : 2.0 µg. Other conditions as in figure 1.

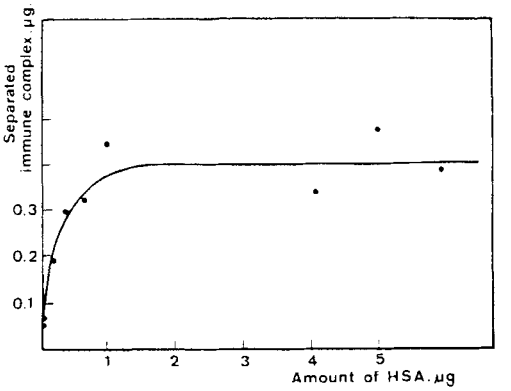

FIGURE 8 : Variation of the amount of the separated immune complex with HSA concentration, at constant Mab HIV anti gp 120.
Injected Mab anti gp120 : 2.0 µg.

PROPOSITION OF TREATMENT TO IMPROVE THE IMMUNE RESPONSE

The difficulty to develop an effective vaccine against certain pathogens can be due to the similarity existing between their antigenic determinants and those of the self antigens of the host. The T cells with receptors recognizing the epitopes common to the two species have been deleted during their maturation in the thymus (negative selection) and there is only a weak immune response following infection, corresponding to the determinants which are recognized as foreign. This response is sometimes insufficient to eradicate the pathogen.

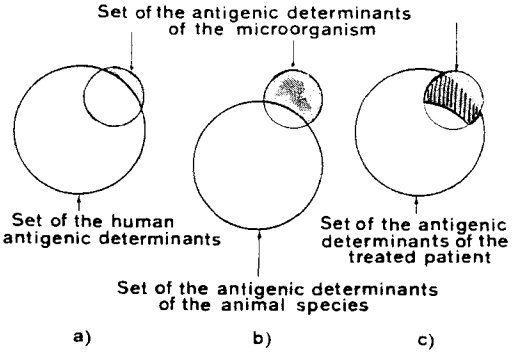

FIGURE 9 : Proposition to improve the immune response.
a) When a pathogen shares the most of its antigenic determinants with those of humans, a small number of antibodies are produced (corresponding to the foreign part, in grey). b) The animal species which is chosen produces a great number of different antibodies (corresponding to the part in grey). They are purified by affinity chromatography and injected into the thymus. c) The set of the self antigenic determinants of the patient is reduced by blocking with the antibodies of the animal species (hachured part). A greater number of determinants of the pathogen are recognized as foreign and elicit antibodies (in grey).

However, there is production of neutralizing antibodies when the same pathogen is injected to another species, sufficiently distant from humans, since many of its determinants are then recognized as foreign. These specific antibodies can easily be purified by chromatography, using an affinity column, with the pathogen bound covalently to the matrix.

In order to improve the efficiency of the human immune response, we propose to block, making use of these specific antibodies, a part of the determinants of the host shared with the pathogen, which are expressed in the thymus, on the MHC antigens, and are responsible for the T cell deletion (Fig. 9). By repeated injections into the thymus, of the specific antibodies prepared and purified as above, one might attempt that the new maturating T cells, with receptors corresponding to the pathogen protein epitopes, will escape deletion and that the immune response will be enhanced.

The choice of the animal species used for the preparation of these antibodies will be important : it must be sufficiently distant from humans, to induce in the animal numerous different antibodies against the pathogen, but not too much distant, to avoid important immune response against the xenoantibodies injected to the patient. It is also necessary to choose animals of great size, in order to obtain sufficient amounts of antibodies. Monkeys, but also Sheeps, Goats, Horses, Pigs could be suitable.

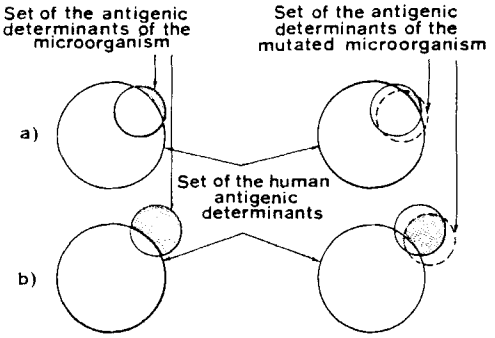

FIGURE 10 : Influence of mutations on the efficacy of a vaccine.
a) The pathogen shares the most of its antigenic determinants with the host. Few determinants elicit antibodies (in grey) and the vaccine prepared with a strain is inefficacious against a mutant. b) Many antigenic determinants of the pathogen are foreign to the host and elicit multiple antibodies (in grey). Even after mutations, a sufficient number of determinants remain unchanged and react with the vaccine prepared with the initial strain.

This reasoning can be applied to the virus of the human immunodeficiency HIV, since its envelope proteins share epitopes with numerous human proteins. By injection of xenoantibodies anti-virus into the thymus, we attempt to make the virus antigenically foreign to the host, by complexing a part of the MHC antigen epitopes which are in common with the virus and are responsible for the deletion of the corresponding T cells. By this means it is expected that the titer of neutralizing antibodies against HIV, which is low or completely missing (11-13), would be enhanced.

One additional difficulty in the preparation of a vaccine against HIV is that the virus undergoes constant antigenic variation, especially in its envelope proteins, from an isolate to the other, even for the same individual (14). In fact, the antibodies are produced against a restricted number of determinants, only those which are recognized as foreign. So that a limited number of mutations can affect these determinants, making the vaccine inefficacious. However, the same number of mutations would be practically without effect if a greater number of epitopes were implied, as it would be the case after the treatment we propose (Fig. 10).

Careful attention must be paid during this treatment to the immune response against the xenoantibodies. In this respect, the choice of the species producing the specific antibodies is essential. When the treatment is stopped, human circulating antibodies against HIV may progressively replace xenoantibodies from their binding sites in the

thymus. A constant watching of the titer of neutralizing antibodies against HIV must be ensured during a sufficient time.

Obviously, a vaccine therapy cannot avoid transmission of the virus by direct cell to cell contact, nor monocyte infection by phagocytose of the immune complexes. However, the validity of the proposed treatment can easily and rapidly be tested with simian immunodeficiency virus SIV infecting Baboons or Rhesus Monkeys.

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