Chromatography of Mast Cell Sensitizing Antibodies in Guinea-Pigs

Mast cell sensitizing antibodies (MCSAb) occur in hypersensitive states and attach themselves in vitro to peritoneal mast cells (MC) which by further exposure to antigen undergo degranulation[1]. This property is the basis of the indirect mast cell degranulation test (IMCD), which has been used for several studies in clinical and experimental hypersensitivity[2-8].

In previous research, we noted that MCSAb from guinea-pigs inoculated in footpads with human albumin (HA) together with complete Freund's adjuvant (CFA) are able to become attached in vitro to both homologous and heterologous MC. (from normal rat), while MCSAb from guinea-pigs inoculated with HA intravenously becomes attached to homologous MC only.

In the present work, we investigated the biological properties and evolution of MCSAb in guinea-pigs following i.v. inoculation of HA, as compared to inoculation in footpads of HA mixed to CFA. The sera were separated on DEAE Sephadex A.50[9] into 3 main fractions (fraction 1, containing the slow moving γ-2-globulin; fraction 2, containing the fast moving γ-2-globulin; and fraction 3, containing the γ-1-globulin), and tested by immunoelectrophoresis against rabbit anti-guinea-pig serum (Sylvana Corp.). Activity of MCSAb, in whole sera (withdrawn at 6, 9, 12, 15, 20 and 30 days after inoculation) and sera fractions, was examined by indirect MC degranulation[2] on guinea-pig and rat MC, and antibody sensitizing homologous skin controlled by passive cutaneous anaphylaxis (PCA)[10]; indirect hemagglutination of tanned red cells, coated with HA, was performed parallel.

Intravenous inoculation of HA was followed by production of a homologous MCSAb present in the γ-1 globulin, together with the homologous PCA antibody (Figure 1); inoculation in footpads of HA mixed to CFA elicited

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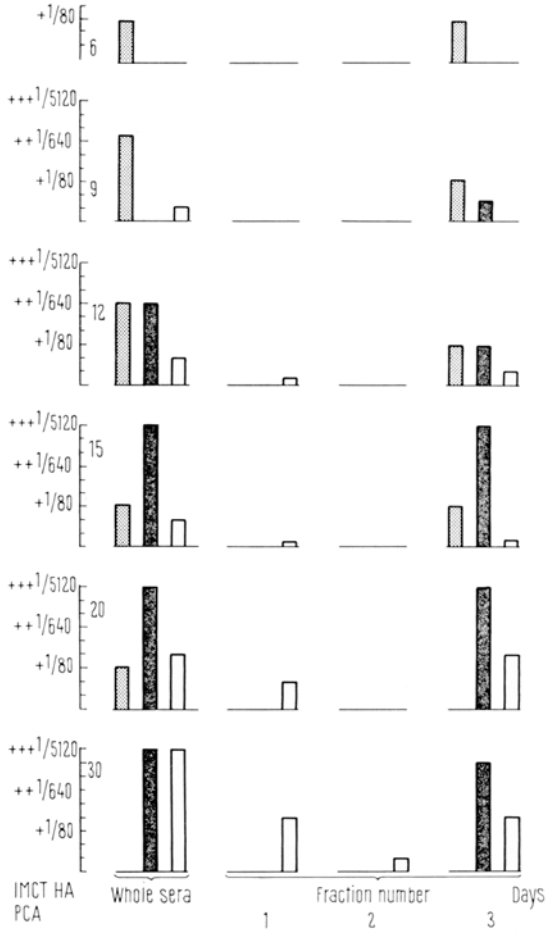

Fig. 1. Evolution of the different antibodies in animals inoculated i.v. with HA. ▨, PCA on guinea-pigs; ▦, PCA on rats; ▩, IMCD test with guinea-pig MC; ▩, IMCD test with Rat MC; □, Indirect hemagglutination.

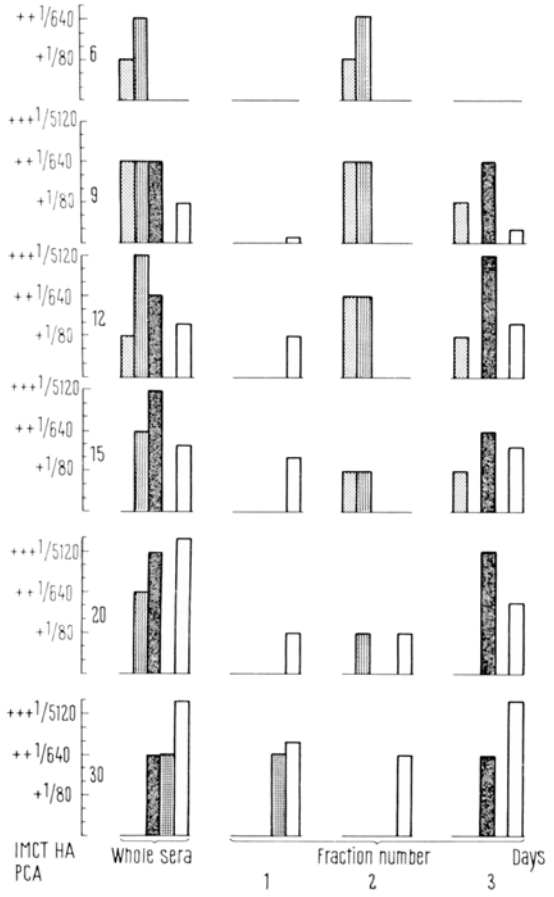

Fig. 2. Evolution of the different antibodies in animals inoculated with HA mixed to CFA, in footpads. ▨, PCA on guinea-pigs; ▦, PCA on rats; ▩, IMCD test with guinea-pig MC; ▩, IMCD test with rat MC; □, Indirect hemagglutination.

formation of a heterologous MCSAb present in the γ-2 fast moving globulin, and of a homologous MCSAb present in the γ-2 fast moving globulin as well as in the γ-1-globulin (Figure 2). In this case, we do not know whether there is a single homologous MCSAb present in 2 different fractions or 2 different homologous MCSAb, 1 in γ-1-globulin identical to the MCSAb, which appears after i.v. inoculation of antigen alone, and the other, which appears earlier and stronger, in the γ-2 fast moving globulin. The homologous skin sensitizing antibody (PCA) occurred, regardless to the type of inoculation, in the γ-1-globulin, and served as control for the accuracy of the separation. The heterologous one (sensitizing rat skin) appeared only after footpad inoculation of HA mixed to CFA; this antibody was found late (30 days after inoculation), and was present only in the γ-2 slow moving globulin. Lack of correlation was noted between evolution of MCSAb, starting early, and disappearing soon after inoculation, and the antibody causing indirect hemagglutination of tanned red cells coated with HA, appearing in the second week following inoculation and reaching its maximum after the disappearance of the MCSAb (Figures 1 and 2).

It seems that the heterologous MCSAb, as well as the homologous MCSAb, present in the γ-2 fast moving globulin, occurs typically after inoculation of antigen together with CFA. This kind of inoculation elicits also the appearance of a heterologous skin sensitizing antibody (PCA). We do not know yet what the relationship is between inoculation of antigen together with CFA and production of heterologous antibodies. Our findings show the sensitivity of the IMCD test, which makes possible the early detection of MCSAb. In some new experiments, we were able to find presence of these antibodies as early as the 4th day after inoculation of antigen together with CFA[11]. Further work is required to establish whether the heterologous MCSAb actually performs a special immunological or biological role in the state following this type of inoculation.

Résumé. L'inoculation intraveineuse d'albumine humaine (HA), provoque chez le cobaye l'apparition d'un anticorps (γ-1) sensibilisant les cellules mast (CM) homologues. L'inoculation dans le coussinet plantaire (HA et adjuvant de Freund complet) fait apparaître deux anticorps: l'un (γ-2) sensibilisant les CM hétérologues, l'autre (dans le γ-1 ainsi que dans le γ-2) sensibilisant les CM homologues.

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Arthus Type Inflammation with Rat Immunoglobulins

Five immunoglobulins classes have been demonstrated in the rat[1-8]. These include IgGa and IgGb, IgM, IgA and γ_1. More recently an additional immunoglobulin, already known as mast cell-sensitizing[9] or anaphylactic[10] antibody, has been identified by radio-immune electrophoresis and termed IgE[11].

IgA and IgE immunoglobulins are usually found in trace amounts in the serum, while the other four classes are largely represented, thus permitting the purification and study of their properties.

The present work was done to compare some of the properties of rat γ_m, γ_1 and γ_2 immunoglobulins (the last one including IgGa and IgGb), and particularly the ability to induce Arthus type reactions in the homologous species.

Materials and methods. Immunization. Wistar male rats were injected in the 4 footpads with a total of 1 ml of emulsion made up of equal parts of bovine serum albumin (BSA) solution containing 4 mg protein/ml saline and

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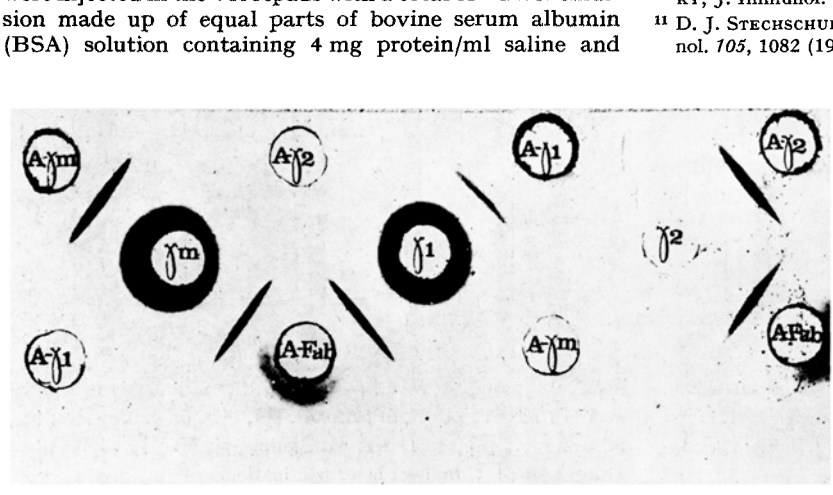

Immunodiffusion analyses of rat γ_m, γ_1 and γ_2 preparations.