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.

N. VARDINON and J. SCHWARTZ[12]

Department of Human Microbiology, Medical School, Tel-Aviv University, Tel-Aviv (Israel), 2 June 1971.

[11] N. VARDINON, in preparation.

[12] We thank Mrs. NAVA RAZ, Mrs. TAMAR MINAI and Mr. I. OFEK for technical assistance.

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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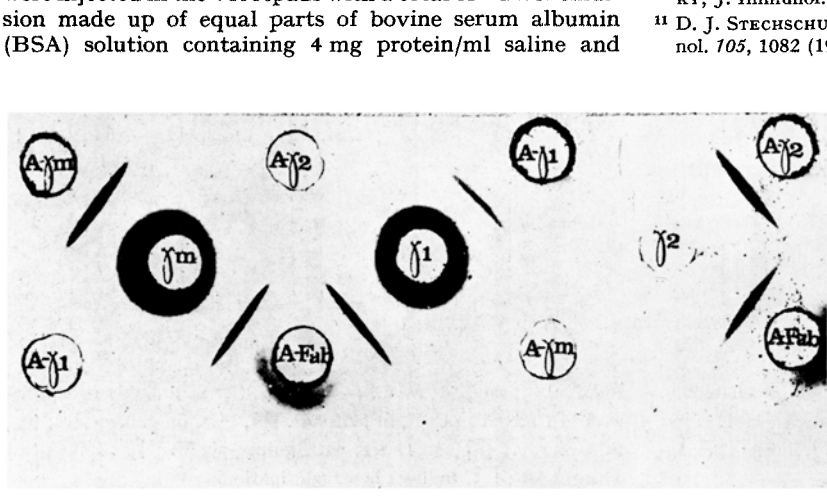

Immunodiffusion analyses of rat γ_m, γ_1 and γ_2 preparations.

Minimum concentration of rat anti BSA immunoglobulins required for immunological reactions[a]

Ig classes	Passive Hemagglutination[b] μg Ab/ml	Passive Hemolysis[c] μg Ab/ml	Reversed Arthus[d] μg Ab/site
γ_m	0.01	0.02	140
γ_2	0.37	2.00	70
γ_1	0.37	2.00	70

[a] The values indicate the antibody concentrations giving: [b] a visible agglutination; [c] 50% lysis; [d] an hemorrhagic spot 5 mm in diameter.

complete Freund's adjuvant. A group of rats were bled 7–8 days after immunization (serum A), a second group of rats were bled 15 to 18 days after immunization (serum B).

Fractionation of rat immunoglobulins. γ_m was obtained from the euglobulin fraction of serum A. The euglobulin fraction was dialyzed against phosphate buffer 0.1*M* pH 8.0 and applied to a DEAE-cellulose column (3 by 60 cm) equilibrated with the buffer. The column was thoroughly eluted with the same buffer and then with phosphate buffer 0.3*M* pH 7.2. The protein peak eluted with the latter buffer is referred to as γ_m fraction.

γ_2 and γ_1 were isolated from serum B by precipitation (twice) with 18% Na_2SO_4 and by stepwise elution of the globulin fraction from DEAE-cellulose using the buffers described by BLOCH et al.[5]. The protein fraction eluted at 0.01*M* pH 7.4 and at 0.05*M* pH 5.0 are referred to as γ_2 and γ_1 fractions, respectively. The concentration of anti BSA antibody in rat immunoglobulin fractions were determined by quantitative precipitin reactions[12].

Antisera specific for rat immunoglobulins. Antisera to rat immunoglobulins were prepared in guinea-pigs as described by BINAGHI et al.[13]. Rat γ_m, γ_2 and γ_1 were obtained from anti BSA antibodies purified according to AVRAMEAS and TERNYNCK[14] and fractionated as above. Guinea-pig antisera were made specific for heavy chains determinants by absorption of immuesera with rat Fab fragment. The Fab fragment was prepared according to NUSSENZWEIG and BINAGHI[2] by papain digestion of rat anti BSA γ_2 antibody.

Immunological procedures. Hemagglutination tests were performed with sheep erythrocytes stabilized by pyruvic aldehyde and formaldehyde and coated with BSA according to HIRATA and BRANDISS[15].

For passive hemolysis, 0.2 ml 1% BSA-coated sheep red cells, prepared using chromic chloride as binding agent[16], were incubated (1 h at 37 °C) with 0.2 ml guinea-pig serum containing 12 $C'H_{50}$ units and $^2/_{10}$ ml volumes of twofold dilutions of anti BSA antibody preparations in veronal buffer[12]. The degree of lysis was estimated from the concentration of unhemolysed cells[17]. The highest dilution that gave 50% lysis was taken as the end-point.

Reversed passive Arthus reactions were induced in Sprague-Dawley rats as reported by COCHRANE et al.[18].

Results and discussion. The concentration of precipitating antibody in γ_m, γ_2 and γ_1 fractions were 740 μg/ml, 1200 μg/ml and 680 μg/ml, respectively. The purity of the fractions was tested by immunodiffusion. In immunodiffusion slides, each fraction showed a precipitin band only when tested with the antiserum specific for the homologous immunoglobulin, ot with rabbit anti rat Fab immuneserum (Figure).

The hemagglutinating, the hemolytic and the Arthus inducing activities of rat immunoglobulins were compared. The results shown in the Table indicate that: a) γ_2 and γ_1 have the same activity; b) the hemagglutinating and the hemolytic activities of γ_m are 37 and 100 times as high as that of γ_2 and γ_1, on a weight basis; c) γ_m is on a weight basis, less efficient than γ_2 and γ_1 to induce reversed Arthus reactions in the homologous species. On a molar basis, however, γ_m is more active than γ_2 and γ_1, considering the molecular weight of γ_m to be 5 times as high as that of γ_2 and γ_1. These results agree with previous reports in the literature[6, 19].

Several authors have investigated the ability of immunoglobulins to induce Arthus type reactions in the homologous species[20, 21]. These experiments, however, have generally been performed with 7S antibodies. The present comparison of the biological activities of rat 19 S and 7 S antibodies demonstrates that γ_m is able to induce reversed Arthus reactions in the homologous species. This finding suggests the possibility that γ_m antibody may be involved in hypersensitivity reactions, expecially during the early steps of immunization. The lower efficiency of γ_m to induce Arthus type reactions may be related to the slow diffusion of γ_m due to its high molecular weight, as suggested by TADA and HISHIZAKA[22]. It can also be related to the low C′ fixation activity of IgM antibody-soluble antigen complexes as compared to IgG antibody complexed with the same antigen[23].

Résumé. Étude comparative des proprietés biologiques des anticorps IgM, IgG_1 et IgG_2 du rat. Les résultats montrent que le pouvoir agglutinant et lytique des anticorps IgM est respectivement 37 et 100 fois supérieur à celui des deux classes d'anticorps IgG. Par contre, en ce qui concerne le phénomène d'Arthus, les anticorps IgM sont moins actifs que les anticorps IgG, si les rapports sont exprimés en poids. Si l'on calcule les rapports par nombre de molécules, les anticorps IgM sont aussi, dans ce cas, plus actifs que les IgG.

G. TOLONE, L. BONASERA and R. S. BRUNO

Istituto di Patologia Generale dell'Università, Corso Tukory 211, I-90134 Palermo (Italia),
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