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Ig-ALLOTYPE–ANTI-Ig-ALLOTYPE REACTION IN RABBIT SKIN

H. FRITZ AND BO HUSBERG

Institute of Medical Microbiology, University of Lund

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SUMMARY

In rabbits immunized against allotype specificities local dermal reactions were produced by intradermal injections of the appropriate allotype antigen. The reactions were characterized by redness of the skin, infiltration and oedema. The reactions initially appeared about 24 hr after the intradermal injections. In fully immunized rabbits they appeared about 4 hr after the injections. The reactions appearing after 4 hr were histologically characterized by perivascular polymorphonuclear leucocyte infiltration, extravasation of blood corpuscles and endothelial swelling. The dermal reactivity could be prevented by pretreatment of the animal with cyclophosphamide. Antihistamine treatment did not suppress the reactivity. The reaction appeared to be of Arthus type.

INTRODUCTION

Our present knowledge of the rabbit Ig allotype system has recently been reviewed by Kelus & Gell (1967). The allotype specificities Aa1, Aa2 and Aa3 reside in the heavy chains of the IgG (Stemke, 1964), IgM (Todd, 1963) and IgA (Feinstein, 1963; Sell, 1967; Lichter, 1967); the specificities Ab4, Ab5 and Ab6, in the light chains (Stemke, 1964). Antisera against rabbit allotypes are prepared by different methods of immunization (Oudin, 1956; Dray & Young, 1958; Dubiski, Dudziak & Skalba, 1958; Dubiski *et al.*, 1959a, b). The antibodies against rabbit allotypes are 7S (Sell & Gell, 1965).

In man anti-Ig's detecting isoantigens of γ-globulin, the Gm markers, are frequently found in cases of rheumatoid arthritis. They are usually IgM. The role of these anti-Igs is still obscure. It was, therefore, considered desirable to investigate the reactions that can be produced by Ig–anti-Ig-allotype interaction. Popielski *et al.* (1966) found that intravenous (i.v.) injection of 10–20 ml of rabbit sera was regularly followed by a fall of the blood pressure and sometimes by death of rabbits previously immunized against the allotype contained by the injected sera. These results were explained by intravascular precipitation.

The present paper describes local reactions to intradermally injected allotype antigens in

Correspondence: Dr Hans Fritz, Institute of Medical Microbiology, Sölvegatan 23, 223 62 Lund, Sweden.

rabbits immunized against these antigens. Attempts were made to find out whether the reactions demonstrated are of anaphylactic, Arthus or delayed type.

MATERIALS AND METHODS

Immunization schedule

Reference allotype sera and antisera were kindly supplied by S. Dubiski, A. S. Kelus and J. Oudin. From a group of fifty rabbits from local breeders, seven rabbits (1800–2500 g) were selected according to the allotypic patterns determined with the Ouchterlony technique. The animals selected were immunized every other week for 3 months with the technique described by Oudin and then once a month with a booster dose. Ovalbumin precipitate (freeze-dry weight about 5 mg) of sera from rabbits immunized with ovalbumin was injected with Freund's complete adjuvant. Antibodies against allotypes Aa1 and Ab6 were produced in four and three rabbits, respectively. No rabbit was immunized against both of these allotypic specificities.

Precipitation in gel

The Ouchterlony technique with LKB standard device. The antigenic sera were used undiluted and diluted 1:2, 1:3, 1:4 and 1:6 in saline. The anti-allotype sera were undiluted.

Intradermal reactions

In rabbits producing anti-allotype antibodies as well as in control animals, areas of the skin were depilated by barium-sulphide ointment. Five to 6 days later sera to be tested were injected intradermally. Tests with 0·2 ml of undiluted sera were performed monthly. At 2–4-month intervals the sera were injected also in dilutions of 1:2, 1:4 and 1:6, respectively, in saline; 0·2 ml was given for each blister. As control, saline and sera not containing the allotype in question were injected at the same time. Allotype serum was also fractionated on a DEAE-Sephadex column with 0·02 M-phosphate buffer, pH 8·2. The protein concentration in the fractions obtained was measured spectrophotometrically at 280 mμ. The protein portions were well separated. Of the contents of the tube with the maximum protein concentration in the IgG portion, 0·2-ml aliquots, undiluted and diluted 1:2, 1:3 and 1:4, were injected intradermally into a rabbit immunized against the allotype in question. The tube contained 4·8 mg protein/ml, determined spectrophotometrically.

Pieces of reacting skin excised at different intervals after the injections were examined microscopially (Dr Måns Åkerman, Department of Pathology, University of Lund).

Anti-Aa1 and anti-Ab6 sera were also injected intradermally into rabbits possessing allotype Aa1 and Ab6, respectively.

Depletion of white blood corpuscles

Three rabbits immunized with allotype Aa1 and with pronounced dermal responses to injections of that allotype were given cyclophosphamide, 50 mg/kg body weight, every other evening until the WBC count was reduced to less than 3000/mm^3. In the mornings of the days when cyclophosphamide injections were to be given the animals received intradermal injections of Aa1-serum and one animal also of non-Aa1-serum. The intradermal injections were continued every other day for 6–8 days after cessation of the cyclophosphamide injections and the WBC count was followed. One rabbit immunized with

allotype Aa1 and given intradermal injections of Aa1 serum every other day without cyclophosphamide served as control.

Antihistamine administration

Antazoline chloride (Antasten®) in a dose of 15 mg/kg body weight was given subcutaneously (s.c.) to two immunized rabbits every ½ hr from 1 hr before the intradermal tests with the relevant allotype used.

RESULTS

Sera from the seven rabbits collected after 4 months' immunization gave heavy precipitation lines in gel diffusion tests with rabbit sera containing the antigenic allotype specificity and against reference sera containing the same allotype specificity. The most distinct precipitation lines were obtained midway between the agar cups when the antigenic allotype sera were diluted 1:3. This was true also for further gel diffusion tests with twenty-eight anti-Aa1 and anti-Ab6 sera, respectively, collected on four occasions from the seven rabbits after booster immunizations.

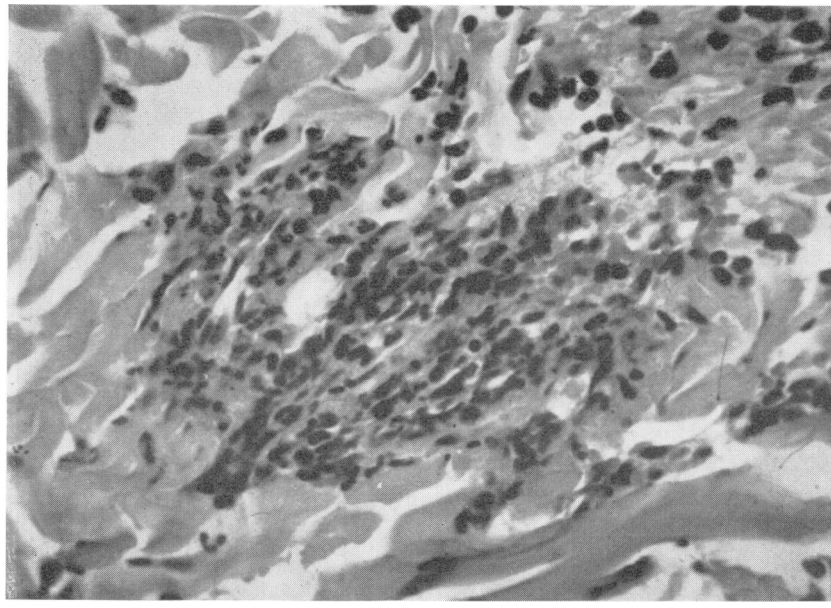

FIG. 1. Perivascular polymorphonuclear leucocyte infiltration in rabbit skin after 6 months' immunization.

The dermal reactions were 16–27 mm in diameter and characterized by infiltration, reddening of the skin and oedema. They were not produced until after 3 months' immunization, e.g. after five to seven injections of antigen. The reactions then appeared 18–26 hr after the intradermal injections, and disappeared about 48 hr later. The reactions were demonstrable with the intradermally injected sera, diluted 1:4. Microscopical examination revealed sparse inflammatory changes. Three months later, after monthly booster immunizations, the reactions appeared after 4 hr and persisted for 48 hr. Histologically these reactions

were characterized by pronounced perivascular polymorphonuclear leucocyte infiltration (Fig. 1), endothelial swelling and extravasation of blood corpuscles in the oedematous tissue. The reactions were never demonstrable with sera injected in dilutions higher than 1:4. From then on, i.e. for a further 10 months, the interval between the injections and the appearance of the reactions as well as the duration of the reactions remained unchanged. There was no difference in the reactions produced by sera containing the allotype specificities Aa1 and Ab6. The same type of reaction, similar also in time of appearance and course, was obtained with IgG preparations of actual allotype specificity diluted to 2·4 mg protein/ml. Sera and Ig preparations not containing the actual allotype specificity produced no dermal reaction. Anti-Aa1 and anti-Ab6 serum injected intradermally into rabbits possessing the allotype in question produced no positive reactions.

Cyclophosphamide treatment of three immunized rabbits reduced the WBC count from, respectively, 11,700, 8300 and 14,200 cells/mm^3 to 2300–2900 cells/mm^3 within 8–14 days. After the beginning of the cyclophosphamide treatment, repeated intradermal injections of the allotype serum in question produced characteristic dermal reactions within 4 hr of the injections until days 6–10 when the dermal response was doubtful. Intradermal injections 2–4 days later, when the WBC count was lowest, produced no dermal response. The WBC count rose within 4 days after the cessation of the cyclophosphamide treatment and within a further 4–6 days the WBC count was normal and positive reactions to the intradermal tests were again obtained. The immunized control rabbit not treated with cyclophosphamide continued to produce positive reactions throughout the experiment.

During antihistamine treatment the intradermal injections of anti-allotype sera produced a dermal response in due time and with ordinary strength.

DISCUSSION

The present study shows that small amounts of allotypic antigen injected intradermally into rabbits immunized with the antigen in question produce local reactions characterized by redness of the skin, oedema and infiltration. The dermal reactions that could be produced after five or seven subcutaneous immunizations appeared about 24 hr after the injection. This result is consistent with a casual observation by Leskowitz (1960) that a rabbit immunized during a short period against an Ig-allotype specificity produced dermal reactions 48 hr after intradermal injections of γ-globulin with the actual allotype specificity. Leskowitz did not prolong the period of immunization.

When the animals in the present study were fully immunized, after three further booster doses, the dermal reactions appeared about 4 hr after the intradermal injections of allotype antigen. In contrast to the dermal reactions after five to seven immunizations these reactions were histologically characterized by heavy polymorphonuclear infiltration and extravasation of red blood corpuscles and thus suggested an Arthus-type reaction. The difference in time of appearance and microscopical picture is hardly ascribable solely to differences in the amount of circulating anti-allotype antibody, because already after the first five to seven immunizations the rabbits produced dermal reactions simultaneously at several sites of injection and with the same dilution of allotype sera, as was found at the time of full immunization. Furthermore, a short time after the rabbits began to show dermal reactivity to allotype antigen, precipitation lines appeared in gel diffusion tests. In later experiments with fully immunized rabbits, the same dilutions of sera gave corresponding gel-precipita-

tion lines of equal position and intensity. It is thus possible that the initial dermal reaction was of delayed type, and that there was a later increase in antibody avidity.

The lack of reaction to intradermal injection of anti-allotypic sera in a rabbit possessing the corresponding allotype may be ascribable to antigen excess.

After suppression of the WBC count with cyclophosphamide treatment, the reactivity of the skin to allotype antigen was abolished in immunized rabbits. The reactivity reappeared when the WBC count increased. Continued intradermal injections of allotype antigens every 2nd day after cessation of cyclophosphamide treatment did not suppress the reactivity. The probable WBC dependence of the skin reactivity is compatible with an Arthus-type reaction as well as with a reaction of delayed type. Antihistamine treatment in large doses did not suppress the skin reactivity to the allotype antigen injected intradermally.

TABLE 1. Interpretation of the experimental results in fully immunized animals, e.g. after about 6 months of immunization

Measurement of study	Accordance with different types of reaction		
	Arthus type	Delayed type	Anaphylactic type
Precipitation in gel	Yes	No	No
Time interval between injection and reaction	Yes	No	No
Microscopic examination	Yes	No	No
Dermal reactivity after cyclophosphamide treatment	Yes	Yes	No
Dermal reactivity after antihistamine treatment	Yes	Yes	No

The interpretation of the results, discussed above, is summarized in Table 1. The results in the fully immunized animals are compatible with an Arthus-type reaction. Although a delayed type of reaction might have initially been involved, the dermal response of fully immunized animals is not consistent with a delayed type reaction. The reaction cannot be of the anaphylactic type.

In preliminary experiments that have been in progress for more than 1 year, injection, every other week, of rabbit sera into knee joints of rabbits immunized against the allotype specificity in the injected sera has so far failed to produce histological or roentgenological changes in the joints.

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