are damaged in these reactions we have no convincing evidence that a similar fate is shared by the lymphocyte.

Runt lymphocytes were regularly cytotoxic to monolayers composed of either runt or normal syngeneic macrophages. This is to be expected since many of the lymphocytes are of donor origin and have been stimulated or sensitised by contact with host tissue. These cells were cytotoxic although perhaps to a lesser degree to monolayers of donor macrophages. Since runt lymphocytes and donor macrophages are most probably syngeneic, any form of specific immunological reaction is unlikely. The explanation may be that stimulated lymphocytes exert a non-specific cytotoxic effect on cell lines in culture (LUNDGREN and MÖLLER[3]).

The runt serum contained an anti-lymphocytic antibody which reacted only with recipient lymphocytes. It caused compact adherence of these cells to macrophage monolayers. Its presence blocked the cytotoxic reaction which occurred when runt macrophages reacted with normal lymphocytes. It is noteworthy that in these instances a cytolytic necrotizing reaction was replaced by one of adherence in which numerous well-preserved macrophages were surrounded by rosettes of lymphocytes. Receptor sites, capable of interaction with the modified surface of the runt macrophage, presumably have been blocked by an antibody in runt serum. This phenomenon may be related to the prolongation of homograft survival in vivo noted by FRENCH and BATCHELOR[4]. Whatever the true explanation our findings give to the macrophage a new role in the pathogenesis of GVH, namely an altered reactivity characterized by cytolytic and adherence reactions on contact with 'self' constituents such as lymphocytes[5].

Résumé. Les macrophages péritoneaux prélevés chez des souris au cours de la réaction du greffon contre l'hôte (RGCH) ont été mis en culture sur des lamelles. Ces cellules sont caractérisées par une plus grande variabilité de taille, une activité de membrane plus prononcée (observée au microscope à contraste de phase) et une avidité plus grande envers des erythrocytes sensibilisés. Le fait principal est que les macrophages des souris RGCH sont auto-agressifs quand ils sont mélangés avec des lymphocytes normaux de souris syngénéiques.

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Soluble Antigen-Antibody Complexes Reactive in Homologous Rabbit Skin

Rabbit homologous skin sensitizing antibodies have been elicited experimentally by the injection of proteins[1], hapten conjugated proteins[2,3], or by infection with various nematodes[4]. They are usually detected 6 days after immunization and disappear within a few days or weeks[2]. More recent reports[5,6], however, indicate that skin sensitizing antibodies may persist for longer periods in rabbit serum and that their characteristics are dependent on the type of immunization. Such homocytotropic antibodies are reported as being associated with γG-[3,5], γA-[7] and recently with γE-like immunoglobulins, the latter occurring in a very small quantity in the circulation of the rabbit after immunization[1,8].

As for human reaginic hypersensitivity reactions, evidence has been presented showing that preformed ragweed-γE antibody complexes give erythema-wheal reactions in normal individuals, whereas the complexes composed of either γG or γA antibody do not[9]. The present study was undertaken in order to test the ability of soluble antigen-antibody complexes to elicit immediate-type skin reaction in normal rabbits. Both 'early' and 'late' antibodies were examined in these complexes. The nature of the antibodies involved in the skin reactions was investigated under different physiochemical conditions.

Randomly bred albino rabbits of either sex, weighing 2.5–3 kg, were used for immunization and reverse type skin reactions. 10 rabbits were immunized with 2 ml DNP-BSA (1 mg/ml) emulsified in complete Freund's adjuvant. The immunogen was prepared by the method of EISEN[10], contained an average of 18 DNP molecules per molecule of BSA and was injected at multiple intradermal sites. A booster i.m. injection was administered 10 and 16 days later. The animals were bled on the 6th, 10th, 16th and 30th day after immunization.

The sera from individual bleedings were pooled to produce a 6th day serum, 10th day serum etc. Quantitative precipitin test[11] of these antisera with DNP-ovalbumin revealed 0, 0.3, 1.1 and 1.9 mg/ml respectively of precipitating antibodies. Globulins were separated from immune sera by precipitation at 4 °C with ammonium sulfate (50% saturation); they were then washed twice with 50% saturated ammonium sulfate, dissolved in distilled water and dialyzed against 0.15 *M* NaCl. Reduction and alkylation of globulin samples was done as previously described[3]. Heat treatment were carried out at 56 °C for 7 h in a shaking water-bath. Succinillation of the globulin fractions was done according to RIVAT et al.[12] using 10% succinic anhydride.

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Preparations containing non-precipitating antibodies were obtained by serial absorptions of precipitating antibodies from native and treated DNP-BSA globulins in the following way: To 1 ml samples of the 0.5% solutions of globulin fractions was added 0.05 ml (0.015 mg) of DNP-OVA containing 14 molecules of DNP per molecule of ovalbumin. The mixtures were then incubated for 30 min at 37 °C, centrifuged and the precipitate discarded. This procedure was repeated until no further precipitate was visible. After adding the last portion of antigen, the solutions were kept for 16 h in the refrigerator and the residual precipitate discarded. In the 6th day serum, no precipitate was visable upon adding the first portion of the antigen. To each of the globulin samples, now deprived of precipitating antibodies, was added 0.05 ml of a solution containing 0.015 mg, 0.030 or 0.060 mg of DNP-OVA. The resulting mixtures were regarded as solutions of soluble antigen-antibody complexes.

Prior to testing them for their skin activity, the mixtures were centrifuged for 30 min at $30,000 \times g$ and filtered through millipore filter (0.65 μm). Skin test was performed by injecting 0.1 ml samples intradermally followed by i.v. injection with 2 ml of 2% Evans Blue solution. The extent of the skin blueing, if any, was measured on the inner side of the skin 30 min later.

Although 3 concentrations of antigen were used, only the one resulting in maximum blueing reaction is represented in the Table. The diameters of the skin reaction recorded in the table are each an average of 3 duplicate test run in 3 different rabbits. The findings indicate that the preformed soluble antigen-antibody complexes are capable of evoking the blueing reaction both at an 'early' or a 'late' stage of immunization. However, the soluble complexes formed with globulin after 16 and 30 days of immunization showed a lower capacity for eliciting the skin reaction. Reduction as well as heat treatment at 56 °C had greater effect on the 'early' antibodies than on the 'late' ones. Succinillation of the globulins slightly reduced the skin reacting capacity of the 'early' antibodies but significantly enhanced the skin-reacting capacity of the 'late' antibodies. Control sites in which antigen alone or antibody alone was injected, gave practically negative results. No measurable blueing was obtained when Evans blue was injected into rabbits 5 h following the intradermal injection of the antigen-antibody complexes.

Preformed antigen-antibody complexes have been shown to induce increased permeability of guinea-pig skin capillaries[13]. Such skin-reactive complexes in guinea-pigs are formed only with the skin-sensitizing antibodies. COOKE et al.[14] found that the injection into normal skin of a mixture of ragweed pollen extract with serum of atopic patients, induced erythema-wheal reactions.

Skin reaction produced by intradermal injection of soluble DNP-OVA-anti-DNP complexes into rabbits

Treatment of the anti DNP-BSA globulin	Intensity of skin reaction (diameter in mm)			
	Days after immunization			
	6	10	16	30
No treatment	21	20	10	12
56 °C 7 h	6	7	8	9
Reduction-alkylation	4	3	6	6
Succinillation	18	17	17	18
Anti DNP globulin	2	3	2	3
DNP-OVA	–	–	–	–

ISHIZAKA and ISHIZAKA[9] established that the formation of skin-reactive complexes is characteristic of γE-antibodies. To rule out the possibility that the injection of soluble antigen-antibody complexes into the skin will produce a new equilibrium which may result in the formation of aggregates and consequent 'aggregated anaphylaxis'[15], we used non-precipitating rabbit antibodies (after serial absorption of the precipitating antibodies) rather than soluble complexes in excess of antigen[16]. It has been shown in previous experiments that preparations containing non-precipitating antibodies retain their skin sensitizing activity[3]. The findings of the present study indicate that the soluble complexes of non-precipitating anti-DNP-antibodies obtained both from 'early' and 'late' stages of immunization, induce immediate hypersensitivity reaction when injected into normal rabbit skin. ISHIZAKA and ISHIZAKA[9] have shown that the reactivity of such complexes is dependent on their composition and especially on their antigen-antibody ratio. Therefore, we examined the skin reactivity of complexes formed in 3 different concentrations, so as to find the one giving maximum reactivity.

The results of reduction-alkylation treatment, heating at 56 °C and succinillation, suggest that the 'early' and 'late' antibodies, although possessing similar homologous skin sensitizing properties, nevertheless differ in their physiochemical properties, since the 'early' and the 'late' antibodies were affected differently by the above-mentioned treatments. Succinillation had little effect on 'early' antibodies but significantly increased the reactivity of 'late' antibody complexes. A possible explanation for this difference may be that the succinillation procedure renders the non-homocytotropic anti-DNP molecules non-precipitating, thus contributing to the formation of soluble complexes which are active in homologous rabbit skin[17].

The question arises as to the mechanism whereby the complexes exert their effect. One possible explanation is that a state of equilibrium is created between randomly associating and dissociating antigen-antibody complexes, whereby free antibody rapidly sensitizes tissues and the observed skin reaction results from the combination of antigen with the tissue fixed antibodies.

Résumé. Des complexes solubles d'antigènes-anticorps préparés à partir de l'anticorps DNP non précipitable de lapin et obtenus à des stades «avancés» et «retardés» d'immunisation produisent une réaction immédiate d'hypersensibilité cutanée quand ils sont injectés dans une peau normale de lapin. La réduction, le chauffage à 56 °C et la succinilation des anticorps suggèrent que les anticorps des stades «avancés» et «retardés» compris dans les complexes diffèrent dans leurs propriétés physicochimiques, bien qu'ils possèdent le même pouvoir de sensibilisation sur les peaux homologues.

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