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BRIEF COMMUNICATION

Binding of Aggregated Human Immunoglobulin to Murine Thymocytes and T Cells Through Receptors for the Fc Region

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Summary. Normal thymus lymphocytes and T cells of mice have the ability to bind heat-aggregated IgG of human origin (aggHIgG), as shown by indirect immunofluorescence. At 4°, the cells bind aggHIgG with an irregular speckled appearance; at 37°, the aggregates are incorporated into the surface membrane, inducing rearrangement of the receptors and capping. At the point of maximum binding capacity, thymocytes show a fairly homogeneous fluorescence pattern, whereas T cells show a heterogeneous appearance. Aggregated pure human Fc fragments, but not Fab fragments, retained not only the binding capacity of the complete molecule but also the ability to induce cap formation on the surface of thymus cells, at 37°.

INTRODUCTION

A receptor for modified immunoglobulin (Ig) was first demonstrated on the surface of B cells by their ability to bind either antigen–antibody complexes (Basten, Sprent and Miller, 1972) or heat-aggregated Ig (Dickler and Kunkel, 1972). Complement does not seem to be involved in this bond, which has been reported to take place through the Fc region of the Ig molecule (Paraskevas, Lee, Orr and Israels, 1972). Recently, an Fc receptor was also found on the surface of both, activated mouse thymocytes, by means of a rosette technique (Yoshida and Andersson, 1972), and activated guinea-pig lymphocytes, using aggregated guinea-pig Ig (Van Boxel and Rosenstreich, 1974). Anderson and Grey (1974), employing a sensitive autoradiographic technique, detected the binding of aggregated mouse IgG by half of normal mouse thymus cells and 85 per cent of mouse spleen cells, but were unable to confirm the bond with isolated Fc fragments or mouse Ig.

In the present study, using an indirect immunofluorescence technique, it has been possible to show that, at 37°, all normal mouse thymocytes and T cells bind human Ig aggregates and that this bond does, in fact, occur via the Fc region of the IgG molecule.

MATERIALS AND METHODS

Animals

Inbred BALB/c mice of both sexes, 4–6 weeks old, were used in most experiments. Mice

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of the AKR, C57Bl, CBA and SWR/J strains were each examined once; similar results to those obtained with BALB/c were found.

Aggregates

Human IgG (Hyland, Travenol Laboratories) was adjusted to a concentration of 1 mg/ml with phosphate-buffered saline and aggregated by heating at 63° for 30 minutes (aggHIgG). For some experiments, the preparations were spun at 50,000 *g* for 1 hour and the supernatant incubated with the cells. Fc fragments of human IgG were prepared by papain cleavage of the molecule. The crude Fc fragments were separated on a DEAE–cellulose resin, equilibrated with 0·1 M dibasic phosphate. Further purification, if necessary, was carried out on a Sephadex G-100 column, equilibrated with 0·1 M phosphate buffer. A pure Fab fragment was kindly provided by Dr M. Reichlin. Purity of the products was controlled by Ouchterlony and by immunoelectrophoresis against rabbit anti-human serum. The Fc fragments were concentrated to 5 mg/ml, the pH adjusted to 7·4, and the suspension aggregated in the same manner.

Preparation of cells

Cell suspensions were obtained as described for the thymus (Santana, Wedderburn and Turk, 1974). Blood was defibrinated and lymphocytes isolated by Ficoll–Hypaque gradient centrifugation. Spleen lymphocytes were also purified by the same method. All cells were thoroughly washed in medium 199. Addition of 5 per cent foetal calf serum did not alter the results.

Varying amounts of aggHIgG were added to 3×10^6 cells. After 40–60 minutes incubation at either 4° or 37°, the cells were washed three times, incubated with the conjugate, sheep anti-human FITC (Wellcome, batch K7247), diluted 1:5, for 30 minutes, washed again twice and examined. The fluorescence microscope and filters used were as previously described (Santana *et al.*, 1974).

RESULTS

Lymphoid cells from thymus, lymph node, spleen and peripheral blood, all bound aggHIg. Large aggregates, such as those obtained from heating whole human serum, bound very strongly to the cells, which appeared as hazy fluorescent rings (Fig. 1). Clumps of cells and free aggregates contaminated the preparations. Human IgG gave a much clearer picture. The temperature of the test was important. When the incubations were done at 4°, the aggregates were irregularly bound to the outside of the membrane, and could be seen as fluorescent speckles. At 37°, the aggregates were incorporated into the cell membrane, inducing mobilization of the receptors and capping.

Systematic studies were then carried out, first using thymus cells and aggHIgG in variable amounts. With low concentrations of aggregates, such as 50 μg, thymocytes began to bind and showed few fluorescent speckles per cell. As the concentration was increased, more aggregates were bound and, at 37°, the speckles formed caps of increasing sizes, until an upper limit was reached. Above this limit, which was 500 μg of aggHIgG, no further increase in the staining of individual cells occurred, but fluorescent aggregates and cell clumps appeared in the preparations. Thymus cells showed an evenly distributed fluorescence, at both their minimum and maximum staining capacity (Fig. 2).

Mixed lymphoid cell populations presented a different picture. At lower concentrations

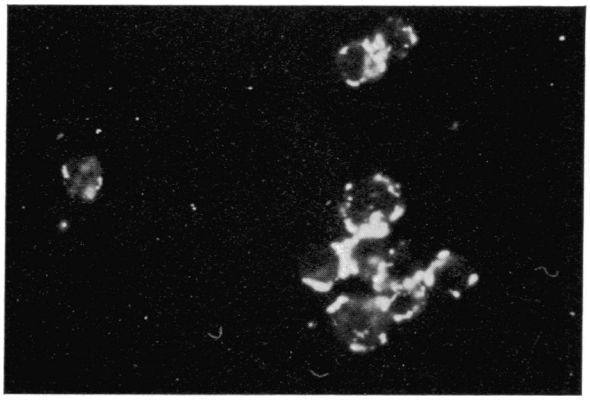

FIG. 1. Clumps of thymus cells stained with aggregated human serum and sheep anti-human FITC. (Magnification ×200).

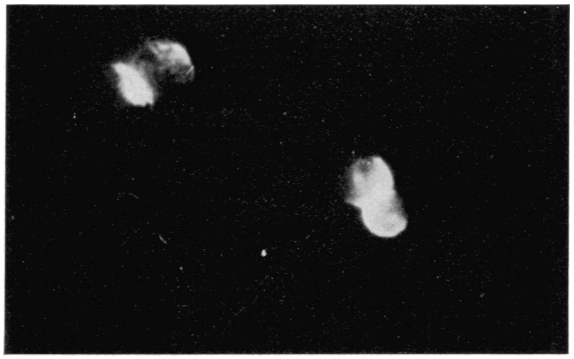

FIG. 2. Thymus cells at the point of maximum staining with aggHIgG, at 37°. Large bright caps, similar to the ones seen on B cells. (Magnification ×200).

of aggHIgG, only a certain number of the spleen, lymph node and blood lymphocytes were able to bind and become fluorescent. With more aggregates, the binding became increasingly greater and only when the receptors on these cells seemed to be saturated, a second population of cells began to stain. Easily detectable surface Ig and cytotoxicity test for θ antigen were also done to characterize these two cell groups. The results, in comparison with the ones found for the thymus cells, are given in Table 1. Saturation of the recep-

TABLE 1

BINDING OF HEAT-AGGREGATED HUMAN IgG TO θ- AND Ig-POSITIVE CELLS OF MICE

	Percentage positive*				
Tissue lymphocytes	Surface Ig (easily detectable)	Agg IgG (50 μg)	θ antigen	Agg IgG (500 μg)	Negative cells
Lymph node	30	30	55	93	6
Spleen	50	52	30	90	10
Thymus	0·2	96	95	95	5

* Mean of 200 cells per preparation.

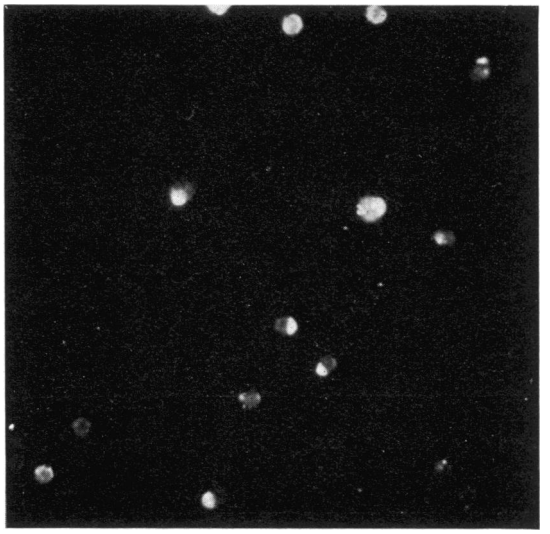

FIG. 3. Lymph node cells at the point of maximum staining with aggHIG. (Magnification × 140).

tors of that second population, which was shown to be θ-positive, was only achieved when using concentrations higher than 1 mg/ml of aggHIgG. At the point of maximum staining, mixed lymphoid cell populations presented a very heterogeneous picture, with both weakly and brightly fluorescent cells (Fig. 3). In any spleen and lymph node cell suspensions, 7–15 per cent of the cells never picked up label.

CONTROLS

Conjugate alone did not stain the cells, ruling out the possibility of a cross-reaction between human and mouse Ig. Supernate of preparations of aggHIgG, spun at 50,000 *g* for 1 hour, had no binding activity. Washing the cells seven, instead of three times, after incubation with the aggregates and before the addition of the conjugate, did not alter the degree of fluorescence; nor did prolonged incubation at 37° for up to 24 hours after the addition of the conjugate.

BINDING OF Fc FRAGMENTS

Even though the experiments referred to above strongly suggested the existence of a receptor for Fc of HIgG on normal murine thymus and thymus-derived lymphocytes, direct evidence to that effect was still missing. For this reason, isolated Fc fragments of HIgG were aggregated by heat and 50 μl of a preparation, containing 5 mg/ml, incubated with the cells. The results showed that aggHFc fragments retained the capacity to bind to the surface of mouse thymocytes and were also able to promote cap formation at 37°. Fab fragments, which were heated under the same conditions, failed to show any binding activity.

DISCUSSION

The results emerging from these studies further support the evidence that murine

thymus and thymus-dependent lymphocytes possess receptors for aggregated Ig of human origin. They also confirm the prediction that the Ig binds to these cells through the Fc region of the molecule. Isolated Fc fragments of HIgG, after being concentrated and heat-aggregated, retained the same binding characteristics of the complete molecule. Of interest is the inability of isolated heated mouse Fc to bind to mouse thymus cells, as reported by Anderson *et al.* (1974). However, as pointed out in that paper, suitable aggregation of the mouse Fc fragments may not have occurred, while this was easily achieved with the human material.

Thymocytes were found to bind aggregates over the same concentration range and were as strongly fluorescent as B cells. At any particular concentration of aggHIgG, the staining pattern of thymocytes were fairly homogeneous, whereas T cells showed different degrees of staining of individual cells, from poorly to strongly fluorescent, at the point at which their receptors seemed to be saturated. This heterogeneous staining capacity, seen in both spleen and lymph node cells, may reflect differences either in the function or in the degree of maturation of T cells.

The presence of Ig molecules on the surface of thymus-dependent cells has now been demonstrated by a number of authors (Santana *et al.*, 1974; Goldschneider and Cogen, 1973; Moroz and Hahn, 1973; Roelants, Ryden, Hagg and Loor, 1974). Marchalonis, Crone and Atwell (1972), using a sensitive radioiodination technique, have isolated approximately equal amounts of monomeric IgM from the surface of thymocytes and T cells, as from B cells of mice and men.

It has also been demonstrated that B and T cells carry the same number of receptors for plant lectins (Greaves and Janossy, 1972). These observations, together with the ones described in the present paper, stress the possibility that those two types of lymphoid cells may, in fact, be less fundamentally different, in their surface properties, than was originally supposed. The controversy in the matter could therefore be largely explained on the basis that T cells, as pointed out before (Marchalonis *et al.*, 1972; Hogg and Greaves, 1972), probably have a special membrane structure, in which the receptors are not always easily detected, unless ideal *in vitro* conditions are fulfilled.

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Announcements

The Cancer Research Institute, Incorporated, is establishing Annual Awards in Cancer Immunology. To inaugurate the program the Institute seeks this year to honour a number of living scientists whose work is judged to have been most critical in founding Cancer Immunology as we know it today, whether in reference to basic experimental research or to observations of more immediate clinical significance.

Nominations, which should be addressed to Mrs Helen C. Nauts, Executive Director, Cancer Research Institute, Incorporated, 1225 Park Avenue, New York, New York 10028, are welcome, and will be considered by a panel of expert consultants (closing date July 15, 1975).

ERRATUM

WICK, G., ALBINI, B. and JOHNSON, W. (1975). 'Antigenic surface determinants of chicken lymphoid cells. II. Selective *in vivo* and *in vitro* activity of anti-bursa and anti-thymus sera.' *Immunology*, **28,** 305.

p. 305, line 10 and p. 310, heading to Table 5. The genotype of skin allotransplant recipients has been incorrectly printed in both instances as B^8B^8. The correct genotype was B^4B^8 as mentioned in the Materials and Methods section.