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The Binding of the Fc Fragment of Guinea-Pig Cytophilic Antibody to Peritoneal Macrophages

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Summary. Using radioactivity assays and radioautography, it has been confirmed that the majority of the guinea-pig anti-BSA cytophilic antibodies are found in the γ_2 IgG fraction. The cytophilic activity was directly demonstrated to be present in the Fc portion of the IgG molecule. Furthermore, the Fc portion quantitatively accounts for the cytophilic activity of the whole IgG molecule.

INTRODUCTION

Cytophilic antibodies have been defined as globulin components of immune serum which become attached to certain cells in such a way that these cells are subsequently capable of specifically adsorbing antigens (Boyden, 1964). Most guinea-pig and mouse cytophilic antibodies to sheep erythrocytes or to soluble proteins have been shown to be present in the slow (γ_2) 7S globulin fraction of serum by starch block electrophoresis (Jonas, Gurner, Nelson and Coombs, 1965; Berken and Benacerraf, 1966) or by DEAE-cellulose column chromatography (Nelson and Mildenhall, 1968; Gowland, 1968). In guinea-pig immune serum, the cytophilic activity, as shown by the uptake of [^{131}I]HSA by sensitized cells, appears to be present only in the IgG fraction (Blazkovec and Sorkin, 1966).

Earlier workers have demonstrated that both guinea-pig cytophilic antibodies (Berken and Benacerraf, 1966) and mouse cytophilic antibodies (Tizard, 1969), isolated from hyperimmune sera, lose their ability to adhere to macrophages after pepsin treatment. Since pepsin is known to destroy the Fc portion of immunoglobulin heavy chains, it was inferred that the cytophilic activity of the antibodies was a function of the Fc portion of the IgG molecule.

In this paper, using radioactivity assays and radioautography with guinea-pig anti-BSA antiserum, I have confirmed that the cytophilic activity is present in the γ_2 IgG fraction. Furthermore, I have demonstrated directly that not only is this activity present in the Fc portion of the molecule but also that the activity of the Fc portion quantitatively accounts for the activity of the whole IgG molecule.

MATERIALS AND METHODS

Animals

Albino guinea-pigs bred randomly and of both sexes were used. They were fed on a pelleted diet (Crajo, Sydney) *ad libitum*.

Immunization

Guinea-pigs were injected with bovine serum albumin (BSA, crystallized, Armour, Eastbourne, England) emulsified in either Freund's complete or Freund's incomplete adjuvant (FCA or FIA). BSA dissolved in 0·15 M NaCl to 5 mg/ml, was emulsified in an equal volume of adjuvant and injected into the four foot-pads at the rate of 0·1 ml/foot-pad. 10 and 17 days later, the animals were injected intradermally with BSA (0·1 mg in 0·1 ml saline) into each of four sites. The animals were exsanguinated under ether anaesthesia 7 days after the last injection. The serum was separated on the same day and stored at $-10°$.

Isolation of anti-BSA antibodies

Anti-BSA antibodies were isolated from immune sera by an immune adsorption procedure. Ethyl chloroformate aggregated BSA prepared according to the following modification of Avrameas and Ternynck (1967) was used as the immunoadsorbent. BSA (10 g) was dissolved in 400 ml of acetate buffer (pH 5·0, 0·1 M). The pH was adjusted to 5·0 with N HCl and kept at that value with N NaOH during the addition of ethyl chloroformate. After standing at room temperature for 1 hour, the solid was dispersed in 500 ml phosphate buffered saline (PBS) using a homogenizer (Virtis, N.Y.). The insolubilized BSA was washed with PBS until free of soluble protein (OD of final wash $<0·05$ at 280 mμ). The immunoadsorbent was stored at 4° in PBS containing sodium azide (1·5 mM).

The immunoadsorbent (100 mg) was added to 100 ml of guinea-pig hyperimmune anti-BSA serum. The mixture was stirred at room temperature for 3 hours and then kept at 4° overnight. The suspension was then centrifuged and the pellet washed with PBS until the supernatant had an OD of $<0·05$ at 280 mμ. The specific anti-BSA antibodies were dissociated from the immunoadsorbent with glycine–HCl buffer, pH 2·2, 0·2 M (4°, 10 minutes). After rapid centrifugation (4°) to deposit the immunoadsorbent, the supernatant was quickly neutralized with 3 N NaOH and dialysed against distilled water overnight at 4°. The protein concentrations were estimated from the OD measurement at 280 mμ in neutral solution assuming $E^{1\%}_{1cm} = 15$ (Nussenzweig and Benacerraf, 1964).

Estimation of antibodies

Antibody contents of guinea-pig hyperimmune sera and purified antibody preparations were estimated by the Farr test (Farr, 1958) and the results were expressed as the antigen (BSA) binding capacity (μg) per ml of serum or per ml of preparation.

Preparation of γ_1- and γ_2-globulins

The specific anti-BSA antibodies were separated into γ_1 and γ_2 fractions by chromatography on a DEAE-cellulose column (Oettgen, Binaghi and Benacerraf, 1965).

Preparation and isolation of Fc and Fab fragments

The purified γ_1 and γ_2 guinea-pig anti-BSA antibodies, obtained from the DEAE-cellulose column, were digested with papain as described by Porter (1959). The protein was dissolved in phosphate buffer (pH 7·6, 0·1 M) containing NaCl (0·005 M), cysteine (0·01 M) and EDTA (0·002 M) to 10 mg/ml and digested with 2 per cent of its weight of papain (Worthington, New Jersey). The digest mixture was incubated at 37° for 4 hours, centrifuged and dialysed against phosphate buffer (pH 7·6, 0·001 M) at 4°. When γ_1

antibody was digested, Fcγ_1 crystallized out on dialysis. The crystals were isolated by centrifugation and washed several times at 4° with small amounts of the same buffer. Fcγ_2 did not form crystals on dialysis but was separated from Fabγ_2 by repeatedly adsorbing Fabγ_2 from the digest with aggregated BSA. Fabγ_2 was recovered from the immunoadsorbent with glycine–HCl buffer (pH 2·2, 0·2 M, 4°). Fabγ_1 was also recovered from the γ_1 digest by adsorption and elution from the immunoadsorbent.

Medium

Experiments involving cells were carried out in the following medium: 100 ml of medium 199 (10 × concentrated, Commonwealth Serum Laboratory, Melbourne), 10 ml of heparin (10,000 units), 50 ml of normal guinea-pig serum, 1 ml of NaN_3 (1·5 M) and made up to 1 litre with PBS. Normal guinea-pig serum used at this concentration did not interfere with the adherence of antibody to macrophages but gave more reproducible results compared to medium without serum.

Peritoneal cells

Normal adult guinea-pigs were anaesthetized with ether, exsanguinated and the peritoneal cavity flushed with 50 ml of medium. The cells were collected in a prechilled beaker, pooled, washed three times with cold medium, and resuspended at $1{\cdot}0 \times 10^7$ cells per ml in medium. The yield was usually $1{\cdot}5 \times 10^7$ cells per guinea-pig and 50–60 per cent of the cells were macrophages as judged by morphology.

Indirect cytophilic antibody assay

Guinea-pig peritoneal cells (5×10^6) in 0·5 ml medium were exposed at 0° for 5 hours to varying dilutions of antiserum or to varying concentrations of the isolated antibody preparations. All dilutions were made in the medium. The reactions, in duplicates, were carried out in plastic tubes (suitable for radioactivity determination). The cells were then centrifuged for 5 minutes at 0°, washed four times in cold medium, resuspended in 0·5 ml cold medium and 200 ng of [^{125}I]BSA in 10 μl PBS containing 1 per cent (w/v) gelatin added. The contents were mixed and left at 4° overnight. At the end of this period, the cells were again washed four times and cell-associated radioactivity was measured by an autogamma scintillation counter (Packard Autogamma Spectrometer). The binding of cytophilic antibody to the cells was expressed as ng of BSA present in or on 5×10^6 cells.

Direct cytophilic antibody assay

The ability of purified γ_1 and γ_2 IgG and of the isolated Fc and Fab fragments to bind to peritoneal cells was tested directly as follows: Each protein preparation was labelled with ^{125}I and centrifuged (90,000 ***g***, 2 hours, 4°) to remove aggregated materials. Varying concentrations of each clarified preparation (100 μl) were reacted at 0°, room temperature or 37° for 1 hour or 4 hours with guinea-pig peritoneal cells (5×10^6) in 0·5 ml medium. The cells were then washed four times in cold medium, resuspended in 0·5 ml cold medium and cell-associated radioactivity rapidly counted. The cells were then smeared on gelatin coated glass slides preparatory for radioautography study. Experiments were carried out in duplicate and the protein bound to 5×10^6 cells was expressed as the percentage of the protein added.

Other methods

Radioautography was carried out as described by Byrt and Ada (1969). Iodination by direct oxidation of carrier free ^{125}I with chloramine T was carried out according to Greenwood, Hunter and Glover (1963) as modified by Ada, Nossal and Pye (1964). The degree of substitution of the [^{125}I]BSA varied from 1 mole of iodide per 17 to 20 moles of BSA and that of the [^{125}I] immunoglobulins and their fragments varied from 1 mole of iodide per 30–50 moles of protein. Polyacrylamide gel electrophoresis was carried out as described by Parish and Marchalonis (1970), and immunoelectrophoresis in agar gel was according to Benacerraf, Ovary, Bloch and Franklin (1963).

RESULTS

PREPARATION OF γ_1- AND γ_2-IMMUNOGLOBULINS

Anti-BSA specific antibodies were isolated from guinea-pig hyperimmune sera using ethyl chloroformate aggregated BSA as immunoadsorbent. The yield of antibody (Farr test) was 30–42 per cent. γ_2- and γ_1-immunoglobulins were separated from the specific anti-BSA antibodies by DEAE-cellulose column chromatography. The yield of antibodies (Farr test) from the column was 75–80 per cent, making an overall recovery of 20–35 per cent. Of the final column eluate, 80–90 per cent could be specifically readsorbed to the immunoadsorbent. Specific anti-BSA antibodies from sera of guinea-pigs injected with BSA in FCA or FIA were fractionated and Fig. 1(a and b) represents the protein profile of the specific antibody fractions when subjected to column chromatography. In each case, two well separated protein peaks were obtained. Each was shown to be a single component

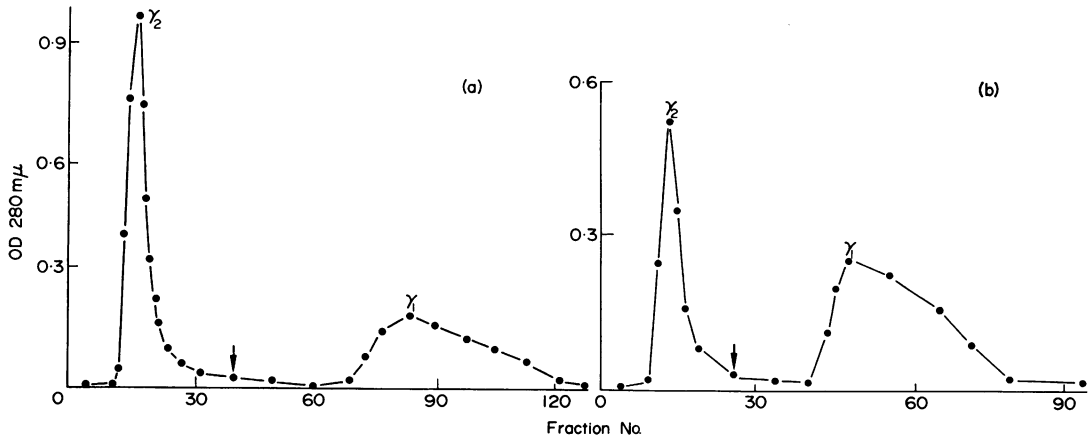

FIG. 1. Separation of γ_2- and γ_1-immunoglobulins from purified guinea-pig anti-BSA antibodies on DEAE-cellulose column with phosphate buffer, pH 8·0. Arrow indicates the starting of buffer gradient 0·01 M to 0·15 M, pH 8·0. (a) purified antibodies from guinea-pigs injected with BSA in Freund's complete adjuvant. (b) Purified antibodies from guinea-pigs injected with BSA in Freund's incomplete adjuvant.

by polyacrylamide gel electrophoresis (Fig. 2) and was identified as γ_2 and γ_1 by immunoelectrophoresis (Fig. 3). In Fig. 3, γ_1 is the faster migrating band. It is interesting to note that sera prepared in either fashion gave rather similar $\gamma_2:\gamma_1$ ratios, being 0·95 in the case of the BSA–FCA experiment and 0·73 in the case of the BSA–FIA experiment.

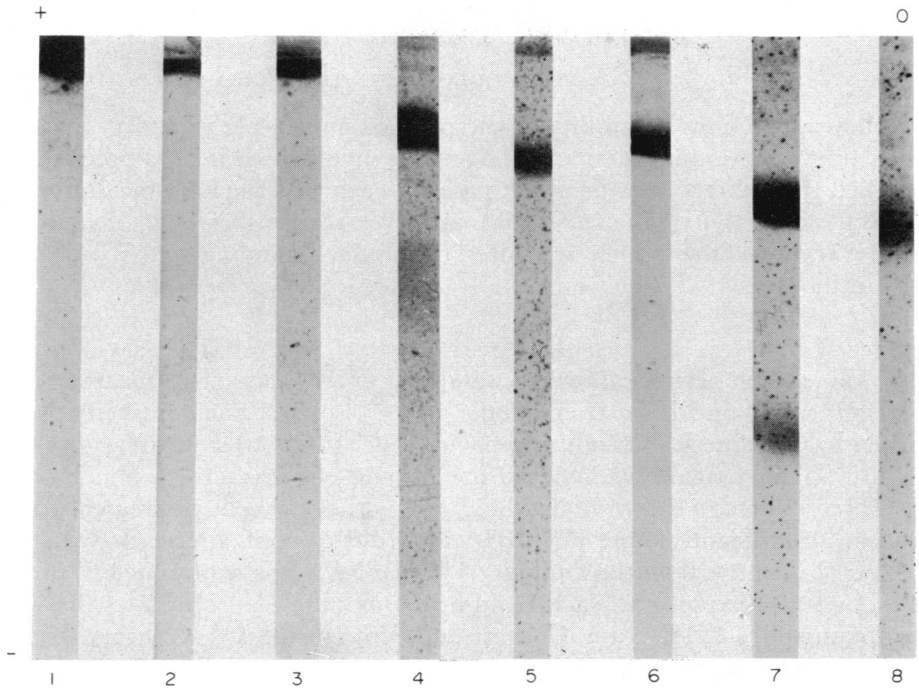

FIG. 2. Polyacrylamide gel electrophoretic patterns of: (1) purified guinea-pig anti-BSA IgG; (2) γ_1 IgG; (3) γ_2 IgG; (4) γ_1 IgG papain digest; (5) Fabγ_1; (6) Fcγ_1; (7) Fcγ_2; (8) Fabγ_2. Both γ_1 and γ_2 IgG (2, 3) show a single band which migrates to a position expected for a 7S globulin. γ_1 IgG papain digest (4) shows no detectable undigested material. Fcγ_1 (6) is from crystallized preparation. The faint band further away from the origin (0) in Fcγ_2 (7) is probably the smaller Fc fragment resulting from further degradation of the fragment by papain.

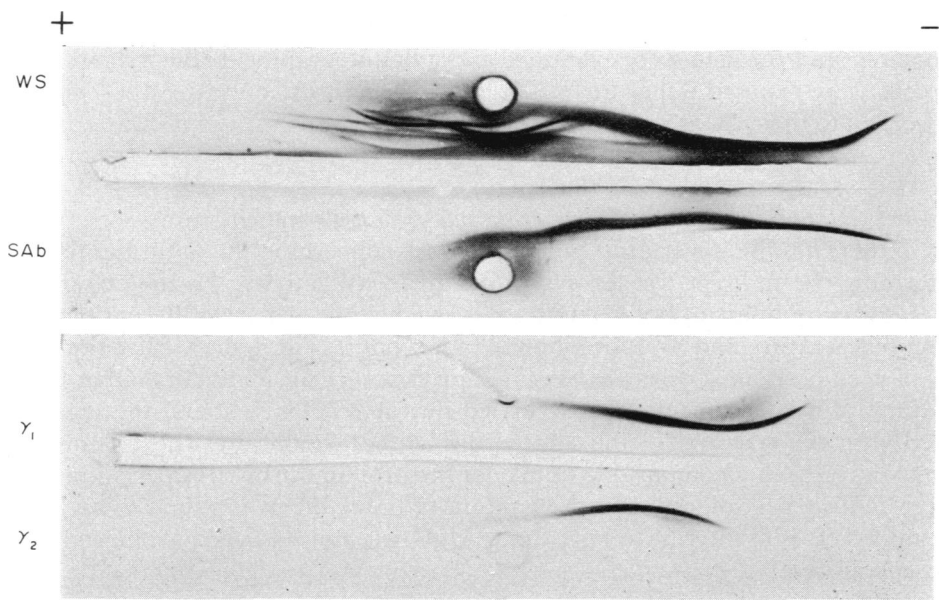

FIG. 3. The immunoelectrophoretic pattern of guinea-pig anti-BSA whole serum (WS) of specific anti-BSA antibodies (SAb) isolated from the whole serum, and of γ_1 and γ_2 IgG prepared from the specific antibodies. The troughs contain rabbit hyperimmune serum against guinea-pig whole serum. Concentrations of protein preparations: γ_1, γ_2, 0·6 mg/ml; specific antibodies, 1·2 mg/ml. The round wells are the points of application of the proteins.

BINDING OF γ_1- AND γ_2-ANTI-BSA IMMUNOGLOBULINS TO GUINEA-PIG PERITONEAL CELLS

In the following sections the ability of the purified guinea-pig γ_1 and γ_2 anti-BSA IgG and of their fragments to bind to macrophages was investigated by two procedures which we have called the indirect and the direct tests. In each case the final measurement is the proportion of radioactivity associated with cells. We realize, however, that the indirect test is the only procedure which measures the antigen binding activity of the intact cytophilic antibodies.

1. *Indirect test*

Normal guinea-pig peritoneal cells containing 50–60 per cent macrophages were exposed to guinea-pig anti-BSA sera or to the γ_1 or γ_2 isolates. Each antibody preparation was adjusted to the same BSA binding capacity (Farr test). After washing, the cells were mixed with [^{125}I]BSA, again washed and the amount of radioactivity bound to the cells estimated. The results are shown in Table 1. The peritoneal cells pretreated with γ_1 IgG did not adsorb significantly more [^{125}I]BSA than did the cells pretreated with medium only and was 55-fold less than the amount of [^{125}I]BSA adsorbed by the cell pre-exposed to γ_2 IgG. Cells pre-exposed to γ_2 IgG adsorbed as much [^{125}I]BSA as did cells pre-treated with anti-BSA–FCA serum. Cells treated with anti-BSA–FIA serum adsorbed less antigen than cells treated with anti-BSA–FCA serum and this probably reflects the different γ_2:γ_1 ratios of the two sera (Fig. 1a and b).

2. *Direct test*

Normal guinea-pig peritoneal cells were exposed to [^{125}I]γ_1 or γ_2 preparations or to [^{125}I]BSA. To minimize pinocytosis, the reactions were carried out at 0° in the presence of sodium azide (1·5 mM). The amount of radioactivity which became associated with the cells was estimated and the cells were examined by radioautography. Table 2 shows that when 5×10^6 cells were exposed either to [^{125}I]IgG or [^{125}I]BSA, only the IgG preparations were adsorbed to the cells at an appreciable extent and of these, γ_2 IgG was adsorbed 10-fold higher than was γ_1 IgG.

3. *The effect of temperature on the binding of γ_1 and γ_2 to peritoneal cells*

There has been some uncertainty in earlier work concerning the optimum temperature for performing the indirect test for cytophilic antibody activity. To investigate this, the direct test for cytophilic antibody activity was carried out at three different temperatures (0°, room temperature and 37°) for 1 hour or for 4 hours. Fig. 4 shows that the maximum binding of γ_2 to peritoneal cells was obtained by exposure for 4 hours at either 0° or room temperature. When the reaction was carried out at 37° for 4 hours, the amount of γ_2 recovered with the cells was of the same order as that found after the 1 hour exposure. There may have been a comparable uptake of the protein during this period but this may have been followed by digestion and excretion of break-down products. This still has to be determined. It is of interest to note that γ_1 IgG was not adsorbed to the cells at any of these temperatures.

4. *Radioautography*

Radioautography (Fig. 5) shows that cells treated with [^{125}I]γ_2 IgG became heavily labelled whilst cells treated with [^{125}I]γ_1 IgG were only lightly labelled. The cells which

TABLE 1

INDIRECT CYTOPHILIC ASSAY OF γ_1, γ_2 IMMUNOGLOBULINS FOR CYTOPHILIC ANTIBODY ACTIVITY

Preparation	BSA binding capacity (μg/0·5 ml)	[^{125}I]BSA added (μg)	[^{125}I]BSA bound to cells* (%)	[^{125}I]BSA bound to cells (ng†)
Normal guinea-pig serum	–	1·0	0·02 (0·01–0·03)	0·2
Anti-BSA–FCA	3·0	1·0	2·2 (1·9–2·4)	22
Anti-BSA–FIA	3·0	1·0	1·1 (0·9–1·3)	11
γ_1 IgG§	3·0	1·0	0·04 (0·03–0·05)	0·4
γ_2 IgG§	3·0	1·0	2·3 (2·0–2·5)	23
Medium	—	1·0	0·01 (0·01–0·02)	0·1

* % of [^{125}I]BSA bound to 5×10^6 normal guinea-pig peritoneal cells. Figures in parentheses refer to range of values of three experiments.

† ng = nanogram = 10^{-9}g.

‡ FCA = Freund's complete adjuvant; FIA = Freund's incomplete adjuvant.

§ γ_1 and γ_2 were samples pooled from both anti-BSA–FCA and anti-BSA–FIA sera as separate test showed that γ_2 prepared from either source were equally active in this test.

TABLE 2

DIRECT CYTOPHILIC ASSAY OF γ_1, γ_2 IMMUNOGLOBULINS FOR CYTOPHILIC ACTIVITY

[^{125}I]preparation	Preparation added to 5×10^6 cells (μg)	Radioactivity bound to cells (%)	Amount of labelled preparation bound to cells (ng)
γ_1 IgG	50	0·33 (0·28–0·35)*	165
γ_1 IgG	100	0·22 (0·20–0·24)	220
γ_2 IgG	50	2·3 (2·1–2·5)	1,150
BSA	50	0·06 (0·04–0·08)	30

* Values in parentheses show range in three experiments.

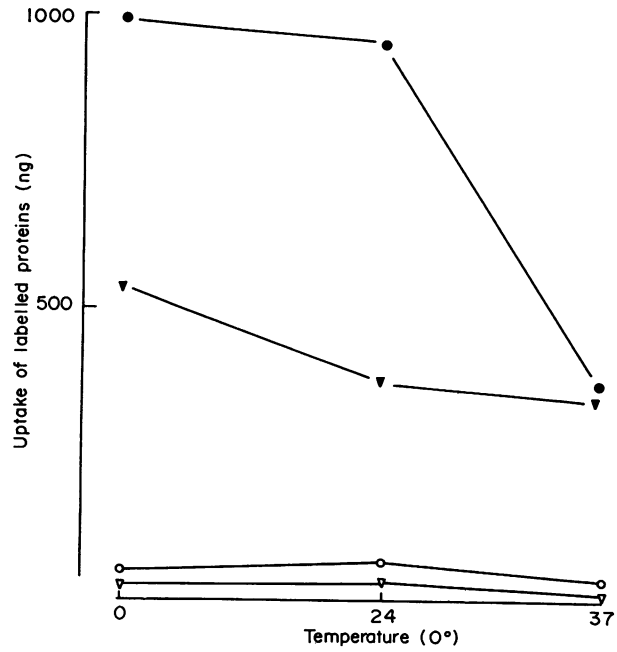

FIG. 4. Effect of temperature on the binding of guinea-pig γ_2 and γ_1 cytophilic antibodies to normal guinea-pig peritoneal cells. 50 μg of [^{125}I]γ_1 or γ_2 in 100 μl PBS was reacted at 0°, room temperature or 37° for 1 hour or 4 hours with 5×10^6 cells in 0·5 ml medium. The cells were then washed four times and cell associated radioactivity counted. The figure represents the result of a typical experiment. γ_2, 4 hours, ●; γ_2, 1 hour, ▲; γ_1, 4 hours, ○; γ_1, 1 hour, ▽.

became heavily labelled upon exposure to [^{125}I]γ_2 IgG were, from morphology, judged to be macrophages. Cells which were judged by morphology to be lymphocytes or polymorphonuclear leucocytes were usually not labelled to an appreciable extent.

ISOLATION OF Fc AND Fab FRAGMENTS FROM γ_1- AND γ_2-IMMUNOGLOBULINS

No trace of undigested immunoglobulin was detectable by polyacrylamide gel electrophoresis (Fig. 2) in the papain digest of the purified γ_1 and γ_2 anti-BSA immunoglobulin.

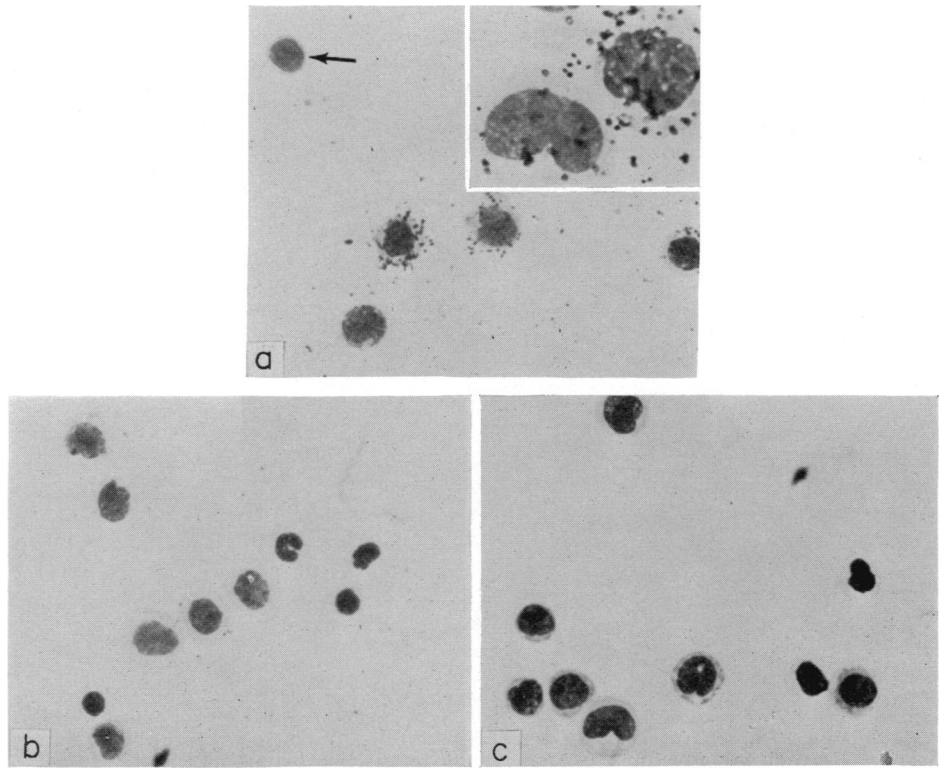

FIG. 5. Radioautographs of normal guinea-pig peritoneal cells after reaction with [^{125}I]proteins. Exposure time was 7 days. × 350. (a) Cells reacted with [^{125}I]γ_2 IgG. The grains can be seen on macrophages but not on lymphocyte (arrow). Insert shows higher magnification (× 840) of some of the labelled macrophages. (b) Cells reacted with [^{125}I]γ_1 IgG. (c) Cells reacted with [^{125}I]BSA.

Whereas the Fcγ_1 was directly isolated by crystallization, the Fcγ_2 could only be obtained as residual material present in the papain digest after repeated adsorption of the digest with the BSA immunoadsorbent. Fig. 6 shows the immunoelectrophoretic pattern of the purified papain digest fragments. In contrast to the other three preparations, the Fcγ_2 preparation shows considerable heterogeneity. To make a more valid comparison of the two Fc preparations, papain digests of γ_1 and γ_2 IgG were both exhaustively adsorbed with the BSA immunoadsorbent. In the case of the γ_1 digest, 75 per cent of the U.V. (280 mμ) adsorbing material was removed from the digest in this way. The corresponding figure of the γ_2 digest was 77 per cent. Following this, the Fcγ_1 was crystallized from the residue of the γ_1 papain digest and examined by immunoelectrophoresis. It can be seen that the isolated Fcγ_1 fragment gave the same pattern as did the residue from which it was crystallized (Fig. 6). There is thus reason to believe that the residue after adsorption of the γ_2 papain digest contains predominantly Fcγ_2.

BINDING OF Fc AND Fab FRAGMENTS TO PERITONEAL CELLS

The Fc and Fab fragments of γ_1 and γ_2 IgG were labelled with ^{125}I and their ability to adsorb to peritoneal cells were tested by the direct method with the results shown in Table 3. Fab fragments from both γ_1 and γ_2 bound poorly to peritoneal cells and to no

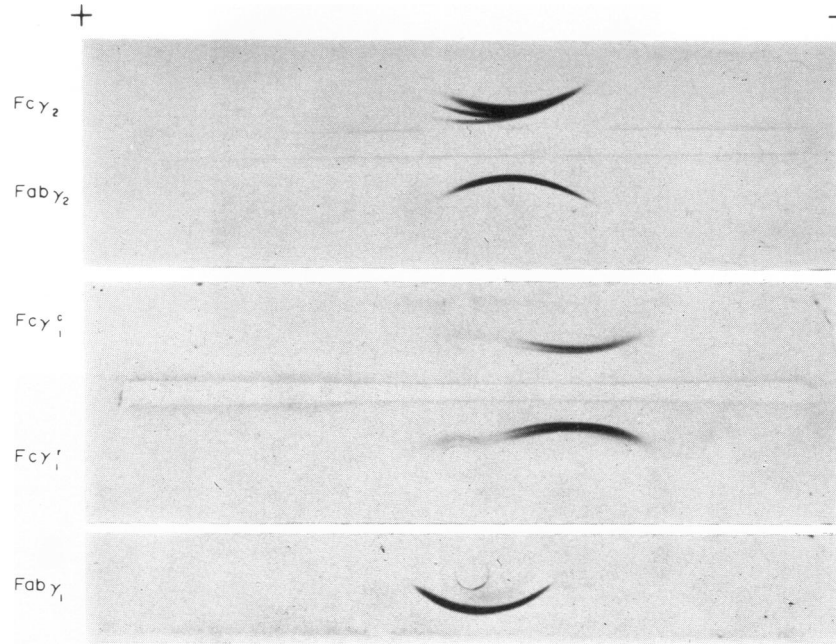

FIG. 6. Immunoelectrophoretic analysis of the fragments isolated after papain digestion of purified guinea-pig anti-BSA antibodies (γ_1 and γ_2). Fc$\gamma_1{}^c$: Fcγ_1 crystallized. Fc$\gamma_1{}^r$: Fcγ_1 residue from γ_1 papain digest after adsorbing out Fabγ_1 with immunoadsorbent. A rabbit antiserum against guinea-pig whole serum was placed in the troughs. Concentrations of proteins: 0·6 mg/ml.

TABLE 3

THE BINDING TO GUINEA-PIG PERITONEAL CELLS OF [^{125}I]γ_1, γ_2 IgG AND OF THE Fab, Fc FRAGMENTS ISOLATED FROM THEM BY PAPAIN DIGESTION

[^{125}I] preparation	Amount added to 5×10^6 cells (μg)	Radioactivity bound to cells (%)	Amount of labelled preparation bound to cells (ng)	μ mole of preparation bound / μ mole of preparation added
γ_1 IgG	32·0	0·14 (0·13, 0·15)*	47	1·48
γ_2 IgG	34·5	2·88 (2·75, 3·01)	992	28·7
Fcγ_1	21·9	0·87 (0·85, 0·89)	190	8·66
Fcγ_2	20·8	2·83 (2·77, 2·89)	582	28·0
Fabγ_1	36·9	0·01 (0·01, 0·01)	55	1·48
Fab γ_2	15·3	0·01 (0·005, 0·02)	14	0·90
BSA	50·0	0·06 (0·03, 0·09)	30	0·60

* Figures in parentheses are individual values.

greater extent than did BSA. Fcγ_1 bound rather better (eight times) to peritoneal cells than did either of the Fab fragments, but only about one-third as well as did the Fcγ_2 fragment. When expressed on a molar basis, Fcγ_2 bound to the cells as well as γ_2 IgG.

Radioautography confirmed these results. Heavily labelled cells were found in cell populations treated with [^{125}I]Fcγ_2 (Fig. 7a) whereas cells treated with [^{125}I]Fcγ_1 were more lighly labelled (Fig. 7b). Cells treated with [^{125}I]Fabγ_1 and [^{125}I]Fabγ_2 were very poorly labelled (Fig. 7c).

TABLE 4

A COMPARISON OF THE CYTOPHILIC ACTIVITY OF γ_1, γ_2 IgG AND THEIR Fc FRAGMENTS

Preparation	Relative cytophilic activity*
γ_2 IgG	47·8
γ_1 IgG	2·5
Fc γ_2	46·6
Fc γ_1	1·4
BSA	1·0

* Relative cytophilic activities are derived from the last column of Table 3, taking the value for BSA as 1·0.

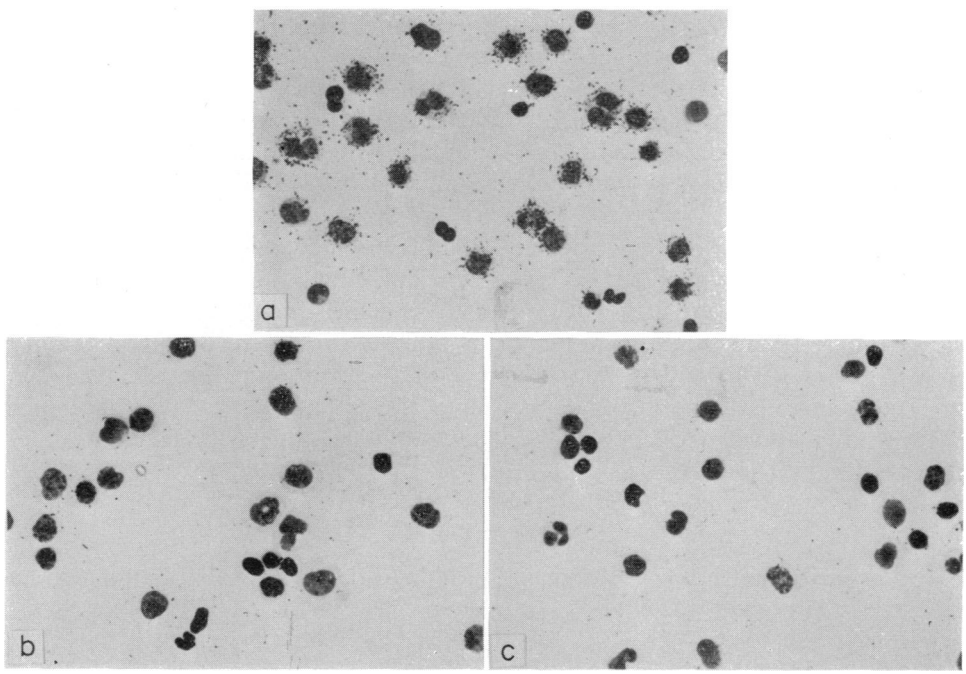

FIG. 7. Radioautographs of normal guinea-pig peritoneal cells after reaction with [^{125}I]fragments isolated from papain digests of purified anti-BSA antibodies (γ_1 and γ_2). Exposure time 7 days. ×350. (a) Cells reacted with [^{125}I]Fcγ_2. (b) Cells reacted with [^{125}I]Fcγ_1. (c) Cells reacted with [^{125}I]Fabγ_2.

DISCUSSION

The results reported in this paper confirm the following findings of earlier workers. 1. Most cytophilic activity in hyperimmune guinea-pig serum is recovered in the γ_2 IgG fraction. 2. This activity is directed towards macrophages and not towards lymphocyte like cells or polymorphonuclear leucocytes (Parish, 1965; Howard and Benacerraf, 1966). 3. Digestion of the γ_2 IgG with papain yields Fab fragments which are unable to bind to macrophages (Berken and Benacerraf, 1966; Tizard, 1969).

Previous workers showed that cytophilic activity could be obtained in guinea-pigs only if the antigen, sheep red blood cells (SRBC), was injected with FCA (Boyden, 1964; Berken and Benacerraf, 1966) and I have confirmed this. However, when BSA was used as antigen, injection into guinea-pigs with FIA also caused the production of cytophilic antibodies. Furthermore, in experiments to be reported elsewhere a similar finding has been made using other antigens (Liew, in preparation).

The principal findings of this paper, as shown by radioactivity counting and radioautography, are two-fold. 1. The Fc fragment isolated from γ_2 guinea-pig IgG has cytophilic activity. 2. This Fc fragment is as active, on a molar basis, as the intact γ_2 IgG molecule. These findings, taken together with the demonstration that the Fab fragment of the molecule has no detectable cytophilic activity, show that the cytophilic activity of the intact molecule is a function solely of the Fc portion of the molecule. The isolated Fcγ_1 fragment also appears to have, in contrast to the intact γ_1 IgG molecule, an appreciable amount of cytophilic activity (see Table 4). However, in this case, radioautography of the smeared cell pellet showed only very light labelling of macrophages compared to macrophages exposed to the Fcγ_2. It is possible that the high count of the cell pellet exposed to the Fcγ_1 preparation may have been due, in part, to some non-specific trapping of the labelled protein. Whether association with antigens affects the cytophilic activity of an IgG molecule is not clear at present because it is difficult to devise a satisfactory method for clearly differentiating opsonizing and cytophilic activity. Parish (1965) was able to distinguish between the two activities to some extent but found a considerable overlap between them.

Cytophilic activity can now be added to those other biological activities which have been shown to be a direct function of the Fc portion of IgG. They are: ability to localize in lymph node follicles (Herd and Ada, 1969), to fix complements (Ishizaka, Ishizaka and Sugahara, 1962), to transfer antibodies from mother to foetus (Brambell, Hemmings, Oakley and Porter, 1960) and to fix antibody to skin (Ovary and Karush, 1961). We are now in a position to look into the mechanism of the binding of the Fc fragment to cell membranes using approaches such as varying the charge of the molecule and by controlled degradation studies.

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