Immunology, 1972, **22**, 821.

Uptake of Antigen by Human Lymphocytes

E. MERLER AND MONICA SILBERSCHMIDT

Immunology Division, Department of Medicine, Children's Hospital Medical Center, and Departments of Bacteriology and Immunology and Pediatrics, Harvard Medical School, Boston, Massachusetts U.S.A

(*Received* 15*th June* 1971)

Summary. Human lymphocytes were fractionated on gradients of bovine serum albumin (BSA). Cells obtained from the more dense layers bound antigen specifically. Cells capable of interacting with antigen contained antibody which was separable with the nuclear components of the cell. Immune cells contained antibody which was specific for the immunizing antigen. Cell-bound antibody mediated and, in the presence of isologous antibody, slightly enhanced the uptake of antigen. Specific antibody passively bound to cell membranes did not promote uptake of antigen. Antigen which bound to lymphocytes *in vitro* was not enzymatically digested within the cell but became bound to acid-insoluble nuclear DNA.

INTRODUCTION

The initial event of the immune response is the interaction of antigen with antigen-reactive lymphoid cells (Davies, Leuchars, Wallis, Marchant and Elliott, 1967). Only a small number of lymphoid cells are antigen reactive and interact with antigen in order that the spleen or a lymph node may subsequently express the reactivity fully (Kennedy, Till, Siminovitch and McCulloch, 1966). Antigen-reactive cells can be fractionated into two populations which, in a density gradient, sediment differently. One of these populations comprises cells that are mitotically active, while the other consists of cells that are mitotically stable (August, Merler, Lucas and Janeway, 1970). Cells in the former class respond to antigenic stimulation by entering into division, while those in the latter take up antigen specifically without subsequent mitotic activity. These mitotically stable, antigen-reactive cells may subserve the function postulated by Miller and Mitchell for trapping antigen (Miller and Mitchell, 1969).

The experiments described herein present evidence that antigen-reactive cells contain antibody. The majority of this antibody is not found on the cell surface. Antigen, after uptake, appears to become bound to nuclear deoxyribonucleic acid (DNA), and may thereby mediate the formation of new cell products.

MATERIALS AND METHODS

Separation of lymphocytes. Tonsils and adenoids were surgically excised for tonsillar hypertrophy from children who had been immunized against diphtheria and tetanus toxoid at intervals of several months or years prior to operation. Tissues were obtained immediately following excision. Lymphocytes were freed from the tissues by cutting with fine scissors,

filtered through glass beads, and washed in medium 199 (without serum) (Merler and Janeway, 1968).

Fractionation of cells. Cells suspended in 17 per cent bovine serum albumin (BSA) solution were layered on top of a discontinuous BSA gradient and separated by centrifugation at 900 ***g*** for 30 minutes. Each gradient was formed by layering BSA solutions (1·5 ml each) of decreasing concentration in a heavy-wall test tube. Concentration limits of 35 per cent and 17 per cent BSA in nine steps were made: each gradient contained 5×10^8 cells (Dicke, vanHooft and vanBekkum, 1968). The cells in each layer following centrifugation were counted in a Coulter counter. The reproducibility of fractionation among gradients assessed by measuring peaks of mitotic activity to phytohaemagglutinin (August *et al.*, 1970) was within one fraction, so that differences of only two fractions or more were considered significant.

Cell disruption by N_2 cavitation. Cells were suspended in a buffer solution having a pH of 7·4 (μ 0·15) that contained sucrose (0·25 M) and $MgSO_4$ (2×10^{-4} M), and washed twice. The cells were equilibrated in an atmosphere of N_2 gas (850 psi for 20 minutes at 4°) while the suspension was stirred. Gas pressure was quickly reduced to atmospheric pressure, and the disrupted cells were collected in disodium ethylene-diamine tetra-acetic acid (EDTA) (final concentration 2×10^{-4} M). Nuclei which had ruptured during this procedure were removed by filtration through a fine nylon mesh. Intact cell nuclei were sedimented by centrifugation at 900 ***g*** for 15 minutes, mitochondria at 22,000 ***g*** for 20 minutes, microsomes and plasma membrane fragments at 100,000 ***g*** for 90 minutes. Four fractions were obtained (Ozer and Wallach, 1967). Generally, preparations in which excess disrupted nuclei were present were discarded.

Incubations of cells with antigen and miscellaneous procedures. Incubation of cells with radiolabelled antigens (1–12 hours) was done in plastic tubes on a rotating wheel at 37° in medium 199. Sodium azide (0·001 M) was added to the cell suspensions, but antibiotics were omitted. Following incubation, cells were separated from the supernatant fluid by centrifugation, and washed four times in medium 199. Radioactivity remaining in cells (uptake) was assayed. Labelling of proteins used as antigens in these experiments with $Na^{125}I$ or $Na^{131}I$ was performed by a modification (Reif, 1966) of the method described by McFarlane. Radioactivity was assayed in a NaI crystal scintillator in a gamma spectrometer. Quantitative γG immunoglobulin determinations were done by radial immunodiffusion in agar (Mancini, Carbonara and Heremans, 1965), using commercial plates but laboratory-prepared standards. Concentration of gamma globulin was adjusted so that samples contained at least 1 mg/ml of γG globulin.

Iso-ionic points were measured in a freshly prepared solution of the protein following elution from Dowex 50 and Dowex 1 resin (Dintzis, 1953).

Concentration of protein was measured in a synthetic boundary cell in the analytical ultracentrifuge by the use of interference optics, assuming that a solution of protein containing 1 g/100 ml corresponds to 40 fringes (Richards and Shachman, 1959) or alternatively by the phenol reaction described by Lowry, Rosebrough, Farr and Randall (1951).

Viability of cells was estimated by determining the proportion of cells able to exclude Trypan blue. Cell preparations which showed a viability of less than 82 per cent were discarded; all cell fractionation experiments were therefore performed on cells viable by this criterion.

RESULTS

DISTRIBUTION OF CELLS AND DEFINITION OF SPECIFICITY

It has been shown that uptake of antigen by lymphocytes correlates with the immunological status of the cell donor (August *et al.*, 1970). Only antigens which, upon incubation with unfractionated lymphocytes, stimulated the incorporation *in vitro* of ^{3}H-thymidine into DNA, were found to be appreciably taken up by the cells. Incorporation of radioactively labelled antigen decreased when labelled antigen was diluted with unlabelled antigen, and was inhibited when unlabelled antigen was incubated with the cells prior to their exposure to labelled antigen. Incorporation was not affected by prior incubation or simultaneous incubation of the cells with an antigen to which the cell donors were not immune. It was shown, therefore, that uptake of antigen was immunologically specific.

Cell counts showing the distribution of lymphocytes in nine albumin layers are shown

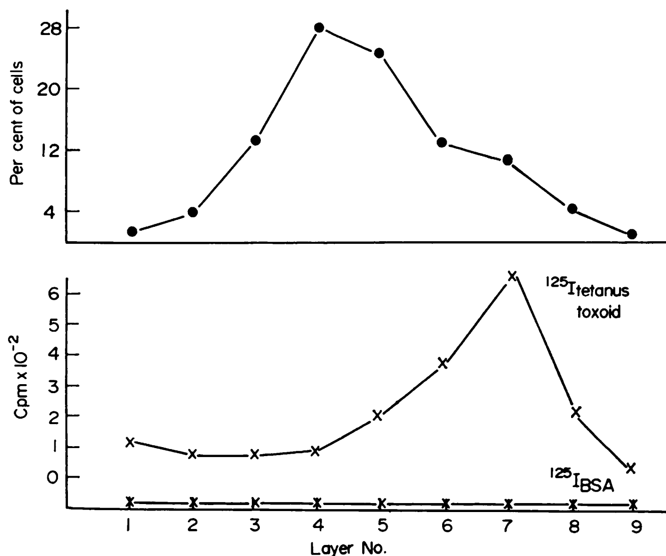

FIG. 1. Fractionation of lymphocytes on a discontinuous density gradient of BSA ranging from 17 per cent (layer 1) to 35 per cent BSA (layer 9). Ordinate: per cent of total number of cells; abscissa: gradient layer. Top: relative distribution of cells. Bottom: uptake of ^{125}I-labelled antigen (tetanus toxoid) compared with control protein (HSA).

in Fig. 1. Incubation *in vitro* of cells from each layer with a radioactively labelled antigen to which the cell donors had been immunized showed that a restricted population of cells incorporated antigen (Fig. 1, lower frame). The amount of antigen incorporated by a constant number of lymphocytes determined from the specific activity of the protein is shown in Table 1.

These experiments confirm previous observations that quantitation of the amount of antigen taken up by a population of cells can be used to identify the immunological status of that population.

The lack of cell donors not immunized with either tetanus or diphtheria toxoids raised the possibility that uptake of these two antigens by the cells could be due to factors other than the immunological competence of the donor. The effect of charge on the uptake of

the protein by the cells was studied. The isoionic point (pI) of several protein antigens is recorded in Table 2. The immunological status of the cells to these two antigens, as defined in the previous section, is also recorded in Table 2. It is seen that in the pH range tested (3–10), only those antigens which had previously been encountered by the cell donors were also recognized by the cells.

QUANTIFICATION OF THE UPTAKE OF ^{125}I-LABELLED PROTEIN BY LYMPHOID CELLS

Uptake of antigens by lymphocytes obtained from the various fractions of the albumin gradient and the distribution of these antigens radiolabelled with ^{125}I in the subcellular

TABLE 1

UPTAKE OF VARIOUS ^{125}I-LABELLED PROTEINS BY LYMPHOCYTES

Protein	Responsiveness of cells*	Weight of protein bound to lymphocytes (μg/10^{10} cells)*
Tetanus toxoid	Immune	5–15
Diphtheria toxoid	Immune	6–12
Botulinus toxoid	Not immune	0·4
Cholera antigen	Not immune	0·6
Pasteurella pestis Fr. 1	Not immune	0·2
BSA	Not immune	0·4

* Values obtained on fifteen different samples.

TABLE 2

MEASURED ISOIONIC POINT (pI) OF VARIOUS PROTEIN ANTIGENS AND IMMUNOLOGICAL STATUS OF CELLS

Antigen	Responsiveness of cells	pI
Botulinus toxoid*	Not immune	2·78
Tetanus toxoid*	Immune	4·03
Cholera antigen*	Not immune	4·22
Diphtheria toxoid*	Immune	4·69
Serum albumin (human)	Not immune	5·31
Plague antigen*	Not immune	5·49
Serum γG globulin (human)	Not immune	6·13
Nuclear histone (bovine)†	Not immune	10·16

* Mol. wt assumed to be 10^5.
† Mol. wt assumed to be 2×10^4.

fractions of the lymphocytes were measured. Results were expressed as number of molecules of antigen interacting with one cell. Concentration of antigen was measured from the specific activity of ^{125}I in the cell pellet and converted to number of molecules by using Avogadro's number. Experiments were repeated at a constant concentration of protein but varying cell concentrations. Maintenance of a constant ratio of molecules of antigen bound to each cell at widely varying cell concentrations would be taken to indicate a homogeneous cell population in which all cells interacted with antigen; that is, if 10^{10} cells bound 10 μg of protein and 10^9 cells 1 μg, that cell population would be considered homogeneous in its reactivity to that antigen. However, experimental varia-

tions of at least four-fold were observed in these experiments. These variations may represent either experimental error or lack of reactivity of a portion of the cells with the given antigen.

Lymphocytes from gradient fractions 6 and 7 which had been washed at least four times in medium free of serum were found to bind between 100 and 400 molecules of antigen per cell. After fractionation into subcellular components, this antigen was associated with components of the plasma membrane (cell surface). Regardless of the immunological status of the cells, this number of molecules of antigen was always bound to the cell membrane. In the case of antigens to which the cells were not immune, this protein represented the total amount of protein bound to each cell. In the case of antigens to which the cells were immune, this protein represented less than 5 per cent of the total amount of protein bound to each cell. Immune cells bound between 1×10^4 and 5×10^4 molecules of protein per cell, and over 91 per cent of the antigen bound to *immune* lymphoid cells was separable with the nuclear fraction and was bound to acid-insoluble DNA.

These experiments indicate that immune cells interact with and incorporate antigen into their nuclear DNA, while suggesting that non-immune cells interact non-specifically with the antigen and bind protein at their surfaces.

RELATION BETWEEN LOCALIZATION AND AMOUNT OF γG GLOBULIN AND THE ABILITY OF THE CELL TO RECOGNIZE ANTIGEN

Lymphocytes which fail to bind to glass surfaces (non-adherent lymphocytes) contain γG globulin (Merler and Janeway, 1968). The amount of immunologically intact γG globulin that is found in cells from each of the layers of the albumin gradient is shown in Fig. 2. Two maxima of γG globulin concentration are noticeable, one occurring in layer 4 and the other in 6. These two maxima coincide with those found (August *et al.*, 1970) for cells which optimally incorporate ^{3}H-thymidine into their DNA after antigenic stimulation (layers 3 or 4) or those optimally found to take up antigen (layers 6 or 7). The experiments show that the concentration of γG globulin in the cells parallels the ability of the cells to respond to antigens. Whether it be by replicating themselves or by trapping antigen, potentially active cells contain larger amounts of γG globulin than those that are quiescent. To see if the intracellular distribution of γG globulin between mitotically active and mitotically stable fractions was different, the following experiments were done.

DISTRIBUTION OF γG GLOBULIN IN SUBCELLULAR COMPONENTS

Table 3 shows the distribution of γG globulin in the subcellular fractions of unfractionated lymphocytes. The values cited are those obtained from replicate determinations on nine different samples. Cells obtained from each of four areas of the albumin gradient shown in Fig. 1 (I, albumin layers 1 and 2; II, layers 3, 4 and 5; III, layers 6 and 7; IV, layers 8 and 9) were pooled, and fractionated after N_2 cavitation. The distribution of γG globulin in the nuclear and cytoplasmic fractions is shown in Table 4. Small amounts of γG globulin of the order of 1 or 2 per cent were identifiable in the mitochondrial and microsomal fraction and are omitted from the Table.

These data show that sections II (layers 3, 4 and 5) and III (layers 6 and 7), which comprise approximately 70 per cent of the total cell population, contain γG globulin distributed differently between nuclear and cytoplasmic layers. Mitotically active cells (section II) contain approximately half of the intracellular γG globulin in the cytoplasm,

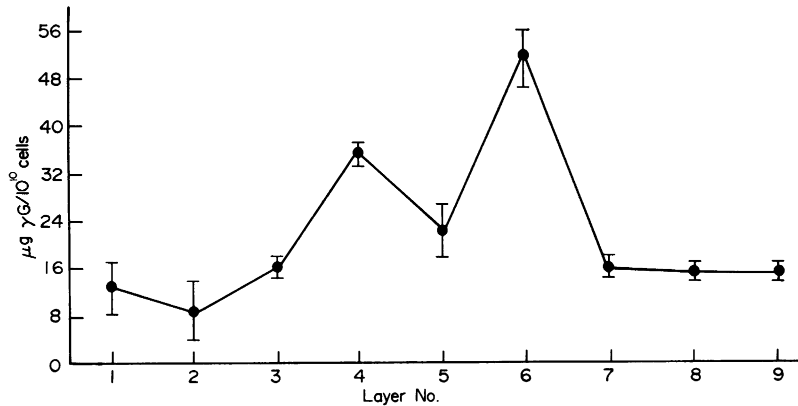

FIG. 2. Content of γG globulin (in μg of γG globulin/10^{10} cells) in cells distributed among the various fractions of an albumin gradient. Replicate determinations on six different gradients of cells obtained from multiple donors.

TABLE 3

DISTRIBUTION OF γG GLOBULIN IN SUBCELLULAR FRACTIONS OF UNFRACTIONATED LYMPHOCYTES OBTAINED AFTER CAVITATION OF THE CELLS BY (a) N_2 GAS AND (b) SONICATION*

Subcellular fractions	Per cent of cell γG
(a) Nitrogen cavitation	
Nucleus	56·5 ± 10·0
Mitochondrion	2·0 ± 0·4
Microsomes and cell wall fraction	2·2 ± 0·4
Supernatant fluid	38·0 ± 6·0
(b) Sonication	
Supernatant solution†	100
Pellet	0

* Replicate values obtained at nine different times.
† 150×10^3 *g* supernate.

TABLE 4

SUBCELLULAR DISTRIBUTION OF G GLOBULIN IN CELLS OBTAINED FROM FOUR SECTIONS OF AN ALBUMIN GRADIENT*

Section	Albumin gradient layer (no.)	Cells (per cent)	G globulin (per cent)	
			Nuclear†	Cytoplasmic
I	1, 2	< 10	—	100
II	3, 4, 5	40	53 ± 10	47 ± 5
III	6, 7	30	82 ± 8	18 ± 4
IV	8, 9	14	50 ± 10	50 ± 5

* Values obtained at five different times.
† Mitochondria, microsomes, and plasma cell membrane fractions, each contained approximately 1 per cent.

while mitotically stable cells (section III) contain over 80 per cent of the intracellular γG globulin bound to the nucleus. These data indicate that morphologically similar, but functionally separable, cells can be distinguished on a chemical basis by the distribution of cell-bound antibody in their subcellular components.

A similar distribution of γG globulin between nuclear and cytoplasmic fractions was observed when cells were disrupted in water, the solution rendered isotonic in NaCl, and the subcellular components separated by fractional centrifugation. While the γG globulin was bound to nuclear materials when the cells were ruptured in water by using two strokes with a glass homogenizer, it was found in the 150,000 ***g*** supernate when the cells were dispersed in water by sonication. The amount of γG globulin found in the 150,000 ***g*** supernate remained quantitatively unchanged when the supernate was added back to a cell suspension dispersed in water with the glass homogenizer and subsequently separated from the nuclear material by fractional centrifugation. At no time were amounts of γG globulin in excess of 1 or 2 per cent of the total found associated with the cell membrane.

Cell lysis by hypotonic shock leaves nuclear DNA highly polymerized and insoluble in dilute salt solutions; DNA-bound components will under these conditions, be pelleted after centrifugation. Sonication, on the other hand, will shear the DNA into small fragments which will be soluble in dilute salt. The experiments described here indicate that binding of antibody to nuclear components is not caused by charge interaction of antibody containing subcellular components with DNA. The experiments indicate that at least part of the γG globulin is associated with the cell nucleus normally, and that it does not become adsorbed non-specifically onto the nuclear membranes during fractionation since cell-derived antibody did not re-bind to nuclear components non-specifically.

UPTAKE OF ^{125}I-LABELLED γG GLOBULIN AND ITS FRAGMENTS BY LYMPHOID CELLS

The uptake of pooled γG globulin fractionated on DEAE cellulose, of Fab and Fc fragments derived from pooled γG globulin, as well as the uptake of γG globulin of subclasses 1–4, is shown in Fig. 3.

Washed lymphocytes bound to their membrane between 100 and 400 molecules of protein. In the case of the Fab fragments, that represented the total amount of protein bound to the cell. The various subclasses of gamma chains appear to bind to the cells in different proportions, although these experiments do not show whether the differences are due to γG subclass or idiotypic specificities. Between 15 and 20 per cent of pooled γG globulin was bound to the cell membrane, and slightly over 70 per cent to the nuclear fraction (in particular to non-histone protein and to acid-insoluble DNA).

Cells from each albumin layer bound approximately equal amounts of γG globulin, its subclasses, or its Fc fragment; for example, 10^3 molecules of Fc fragments were bound to every lymphocyte derived from each layer of the albumin gradient. These experiments show that the uptake of γG globulin by lymphocytes is Fc mediated and that it may be subclass specific. To see if antibody passively bound to lymphocytes would confer on these cells the ability to interact with antigen, the following experiments were performed.

ANTIGEN UPTAKE: EFFECT OF PRE-INCUBATION OF CELLS WITH ANTIBODY

The effect of pre-incubation of tetanus-immune cells with an antibody to tetanus toxoid on the uptake of tetanus toxoid is shown in Fig. 4. Tetanus-immune cells were incubated with an anti-tetanus antibody, washed extensively, and subsequently incubated with

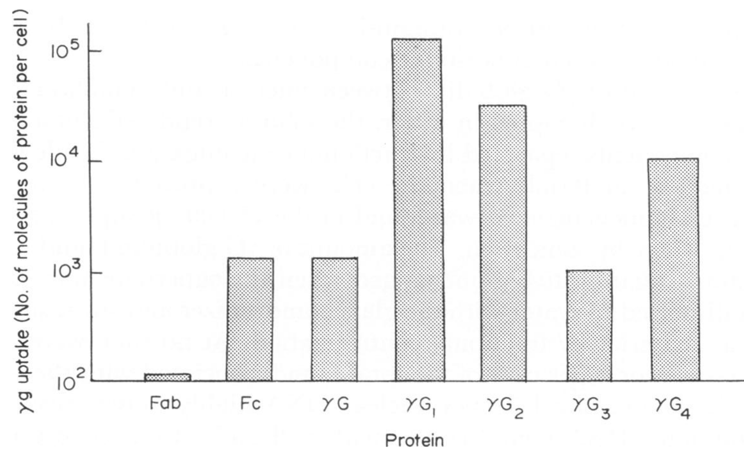

Fig. 3. Uptake of pooled γG globulin, Fab and Fc fragments, and of γG1, γG2, γG3, and γG4 globulins by lymphocytes fractionated in discontinuous albumin gradients. Uptake of each protein was measured for each layer of the albumin gradient at four different times. Only one myeloma protein of each subclass was used.

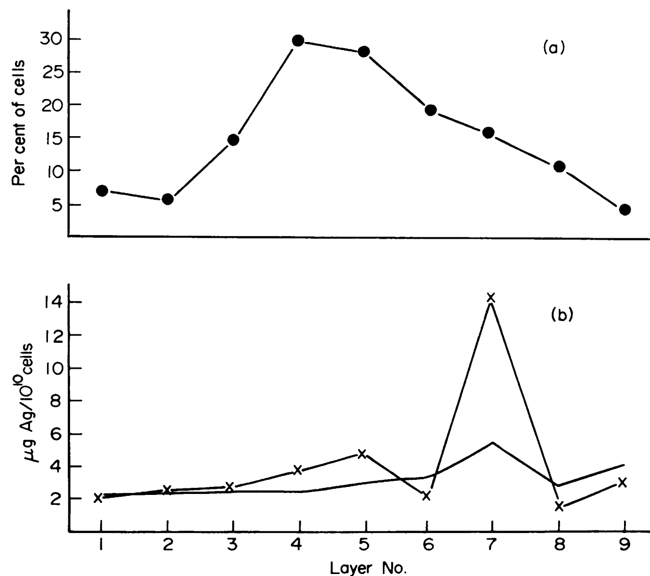

Fig. 4. Fractionation of lymphocytes on an albumin gradient: (a) per cent of cells in each layer of albumin; (b) uptake of antigen (tetanus toxoid) by immune cells in each albumin layer (——); effect of pre-incubation of the cells with an antibody to tetanus toxoid, followed by extensive washing and incubation with antigen (×). Average values of four different determinations.

tetanus toxoid. Aliquots of 10^{10} immune cells that took up 5·2 μg of antigen were found to take up 14 μg of antigen following pre-incubation of the cells with antibody. This increase was specific, was not affected by pre-incubation of the cells with mixed tetanus and botulinus antibody, but was abrogated by pre-incubation with botulinus antibody in place of the tetanus antibody. Since, however, the increase was within the range of values found for the uptake of antigen by immune cells, it is not clear if the increase represented an enhancement of uptake caused by antibody or simply experimental variation. On the other hand, in a second experiment, cells not immune to botulinus toxoid were pre-incubated with botulinus antibody, washed, and subsequently incubated with botulinus antigen. Although containing measurable amounts of botulinus antibody, aliquots of 10^{10} cells took up 0·4 μg of toxoid, an amount identical to that taken up by cells that had not been incubated with antibody. In both non-immune cell populations, those that had been pre-incubated with antibody and those that had not, antigen was bound to the cell membrane, indicating that non-immune cells do not engulf increased amounts of antigen even though they contain antibody. A corollary of this is that the mere presence of antibody on lymphocytes is not sufficient to render a cell immune.

DISCUSSION

In the present communication, an attempt has been made to define the chemical basis of cellular immune specificity by studying the interaction of soluble protein antigens with lymphoid cells. Two principal observations have been made which merit discussion and further inspection. First, uptake of antigen by lymphoid cells is mediated by antibody-like substances which are bound to both the external and nuclear membrane of the cell. Secondly, antigen which has become cell bound internally appears associated with acid-insoluble, nuclear DNA. In these studies, lymphocytes were obtained from human lymphoid tissue (the palatine and pharyngeal tonsils) and were freed from polymorphonuclear cells, macrophages, plasma cells, and those antigen-reactive cells which are mitotically active (August *et al.*, 1970).

Uptake of antigen requires energy since the reaction does not take place in the cold (4°) but proceeds optimally at 37°. The reaction neither depends on cell respiration, since antigen uptake takes place in the presence of Na azide (Cohn, 1966), nor requires complement, since it takes place in a synthetic medium in the absence of serum. The cells that take up antigen do not seem to 'process' antigen by reducing it to smaller fragments, as phagocytic cells do, since nuclear-bound antigen appears by sedimentation in the ultracentrifuge intact following hydrolysis of the nuclear material by deoxyribonucleases. Lymphocytes, therefore, appear to handle antigen differently from macrophages, which are known to degrade and digest (process) antigens with their lysosomal enzymes.

Non-immune lymphocytes kept in a protein-free medium bind foreign proteins reversibly. This suggests that there is no specific chemical interaction between antigen and the surface of the lymphocyte, but rather simple physical adsorption of antigen to the cell surface. This process appears to lack immunological specificity. Proteins adsorbed on the surface of non-immune lymphocytes are excluded from the cell, although the cell membrane, like the placenta, is permeable to Fc fragments of γG globulin. In this way, lymphocytes can incorporate a large number of antibody molecules, irrespective of the immune status of the cell. This passively absorbed antibody appears partly bound to nuclear DNA. It seems to facilitate antigen recognition in immune cells but has no

immunological effect on non-immune cells. Finally, the surface of lymphocytes is permeable to protein antigens to which the cell donors are immune. As many as 50,000 antigen molecules may enter a single cell. Antigen uptake requires energy and the process is not easily reversible, which suggests that there is chemical interaction between antigen and the immune lymphocytes.

These results expand those of many investigators (Ada and Byrt, 1969; Cerottini and Brunner, 1967; Pernis, Formi and Amante, 1970; Rabellino, Colon, Gray and Unanue, 1971; Raff, Sternberg and Taylor, 1970; Wigzell and Anderson, 1969; Wigzell and Makela, 1970) who have presented evidence, in most cases indirect, that immunoglobulin receptors exist on the surface of lymphocytes. It is not clear from the present studies whether the γG globulin separable in small amounts from the plasma membrane fraction of lymphocytes represents, in fact, these immunoglobulin receptors or is simply a contaminant from other subcellular components. It appears, however, that as the cell changes from non-immune to immune status, possible immunoglobulin receptors or structural entities of the cell surface need not change, redistribute, or multiply to accommodate the increased amounts of antigen with which the immune cell interacts, since most of the antigen taken up by the immune cell is bound to the nucleus. There is, in other words, no need to postulate that an immune lymphocyte accommodates a larger number of antigen molecules on its surface than a non-immune lymphocyte.

One major assumption which we have made in obtaining the number of molecules bound to each cell, is that all cells within one gradient fraction react homogeneously with a soluble protein. Some justification for assuming this has been obtained by the cell dilution studies described. The uncertainty inherent in this approach is obvious and has been expressed by the wide range of values described. Specifically, the data fail to distinguish whether approximately 20 per cent of the cells bind 50,000 molecules per cell or all of them bind 10,000 molecules. The dilution studies, however, exclude the possibility that one or two cells bind *all* the antigen. Since the population of cells that take up antigen represents approximately 10 per cent of the total population of non-adherent cells in these studies, it is not clear whether 10 per cent or 2 per cent of the cells are antigen reactive. Attempts at localizing the radioactivity in smears of these cells by microscopic radioautography have not been successful thus far.

The role of cell-bound antibody in uptake of antigen varies, depending on the way in which antibody is bound to the lymphocytes. While passively adsorbed antibody may enhance uptake of antigen in cells obtained from previously immunized donors, it does not render a cell from non-immunized donors sensitive to antigen. γG globulins obtained from cells of donors immune to tetanus and diphtheria toxoids indeed exhibited antibody activity. They did not exhibit antibody activity for antigens to which the cell donors were not immune. It is interesting, in light of these facts, that cell-bound antibodies have never been found in chemical union with antigen during fractionation of lymphocytes following their incubation with radiolabelled antigens. A possible explanation for this fact is proposed by Merler and Silberschmidt (1972).

The presence of a population of cells of bone marrow origin, presumably non-replicating and radiation sensitive, which are essential for trapping antigen, has been postulated by Miller and Mitchell (1969). They assumed these to be the cells that Miller and Nossal (1964) had described as trapping antigen in the splenic follicles and marginal zone. The antigen-reactive and mitotically stable cells described in this communication could, in fact, represent the apparatus postulated by Miller and Mitchell.

The significance for immunological activity provided by the DNA-bound antigen is at present not known; it may presumably either regulate or modify the information contained within a cell and influence its transmission to the cells responsible for the expression of immunological activity.

ACKNOWLEDGMENTS

The authors wish to express their appreciation to the U.S. Department of the Army for the gift of the plague Fraction I, the botulinus, and cholera toxoids; to the Mass. Biological Labs for the tetanus and diphtheria toxoids; to Dr C. G. Flake for the surgical specimens; to Dr N. Abramson and Dr C. Alper for the gift of the myeloma proteins; and to Miss G. Williams and Miss K. Silvennoinen for expert technical assistance. This work was supported by U.S.P.H.S. grants AI-05877 and AI-00366.

REFERENCES

Ada, G. L. and Byrt, P. (1969). 'Specific inactivation of antigen-reactive cells with ^{125}I-labelled antigen.' *Nature (Lond.)*, **222**, 1291.

August, C. S., Merler, E., Lucas, D. O. and Janeway, C. A. (1970). 'The response *in vitro* of human lymphocytes to phytohemagglutinin and to antigens after fractionation on discontinuous density gradients of albumin.' *Cellular Immunology*, **1**, 603.

Cerottini, J. C. and Brunner, K. T. (1967). 'Localization of mouse isoantigen on the cell surface as revealed by immunofluorescence.' *Immunology*, **13**, 395.

Cohn, Z. A. (1966). 'The regulation of pinocytosis in mouse macrophages. I. Metabolic requirements as defined by the use of inhibitors.' *J. exp. Med.*, **124**, 557.

Davies, A. J. S., Leuchars, E., Wallis, V., Marchant, R. and Elliott, E. F. (1967). 'The failure of thymus-derived cells to produce antibody.' *Transplantation*, **5**, 222.

Dicke, K. A., vanHooft, J. I. M. and vanBekkum, D. W. (1968). 'The selective elimination of immunologically competent cells from bone marrow and lymphatic cell mixtures. II. Mouse spleen cell fractionation on a discontinuous albumin gradient.' *Transplantation*, **6**, 562.

Dintzis, H. M. (1953). 'Studies on the dielectric properties of human serum mercaptalbumin solution.' Ph.D. thesis, Harvard University.

Kennedy, J. C., Till, J. E., Siminovitch, L. and McCulloch, E. A. (1966). 'The proliferative capacity of antigen-sensitive precursors of hemolytic plague-forming cells.' *J. Immunol.*, **96**, 973.

Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951). 'Protein measurement with the Folin phenol reagent.' *J. biol. Chem.*, **193**, 265.

Mancini, G., Carbonara, A. O. and Heremans, J. F. (1965). 'Immunochemical quantitation of antigens by single radial immunodiffusion.' *Immunochemistry* **2**, 235.

Merler, E. and Janeway, C. A. (1968). 'Immunochemical identification of cytophilic antibody in human lymphocytes.' *Proc. nat. Acad. Sci. (Wash.)*, **59**, 2013.

Merler, E. and Silberschmidt, M. (1972). 'Separation of antibodies from subcellular components of lymphocytes'. *Immunology* **22**, 813.

Miller, J. F. A. P. and Mitchell, G. F. (1969). 'Thymus and antigen-reactive cells.' *Transpl. Rev.*, **1**, 3.

Miller, J. J. and Nossal, G. J. V. (1964). 'Antigens in immunity. VI. The phagocytic reticulum of lymph node follicles.' *J. exp. Med.*, **120**, 1075.

Ozer, J. H. and Wallach, D. F. H. (1967). 'H-2 components and cellular membranes: Distinctions between plasma membrane and endoplasmic reticulum governed by the H-2 region in the mouse.' *Transplantation*, **5**, 652.

Pernis, B., Formi, L. and Amante, L. (1970). 'Immunoglobulin spots on the surface of rabbit lymphocytes.' *J. exp. Med.*, **132**, 1001.

Rabellino, E., Colon, S., Grey, H. M. and Unanue, E. (1971). 'Immunoglobulins on the surface of lymphocytes. I. Distribution and quantitation.' *J. exp. Med.*, **133**, 156.

Raff, M. C., Sternberg, M. and Taylor, R. B. (1970). 'Immunoglobulin determinants on the surface of mouse lymphoid cells.' *Nature (Lond.)*, **225**, 553.

Reif, A. E. (1966). 'A simple procedure for high efficiency radioiodination of proteins.' *Fed. Proc.*, **25** 725.

Richards, E. G. and Shachman, H. K. (1959). 'Ultracentrifuge studies with Rayleigh Interference Optics. I. General application.' *J. phys. Chem.*, **63**, 1578.

Wigzell, H. and Andersson, B. (1969). 'Cell separation on antigen-coated columns. Elimination of high-rate antibody-forming cells and immunological memory cells.' *J. exp. Med.*, **129**, 23.

Wigzell, H. and Makela, O. (1970). 'Separation of normal and immune lymphoid cells by antigen-coated columns. Characteristics of membrane antibodies as analyzed by hapten-protein antigens.' *J. exp. Med.*, **132**, 110.