Immunology 1976 **30** 695

Separation by column chromatography of cells active in delayed-onset hypersensitivities

H. P. GODFREY *Institute for Experimental Immunology, University of Copenhagen, Norre Alle* 71, 2100 *Copenhagen, Denmark*

P. G. H. GELL *Department of Experimental Pathology, University of Birmingham Medical School, Birmingham*

Received 7 *July* 1975; *accepted for publication* 30 *October* 1975

Summary. Lymph node cells from guinea-pigs contact sensitive to 1-thiocyano-2,4-dinitrobenzene have been fractionated by affinity chromatography over modified polyacrylamide beads. Cells mediating lymphokine release in response to active sensitizer were depleted only by chromatography over dinitrophenyl (DNP) containing substrates and could be specifically eluted with DNP–glycine. DNP rosette-forming cells (RFC) were equally well depleted by chromatography using either DNP or trinitrophenyl containing materials but could not be eluted from the columns by DNP–glycine. While the antigen receptors of cells mediating the release of macrophage agglutination factor in response to DNP-containing antigens and of DNP–RFC were found to be hapten-specific, their specificity was shown to differ using chromatography over trinitrophenyl containing polyacrylamide.

INTRODUCTION

Guinea-pigs sensitized with reactive chemicals in Freund's complete adjuvant (FCA) develop delayed-onset skin reactions both to the sensitizer itself (contact) and to related hapten–protein conjugates (intracutaneous injection) as demonstrated by Gell and Benacerraf (1961). These two reactions have a similar histological appearance including marked infiltration of basophils in the upper dermis (Dvorak, Simpson, Bast and Leskowitz, 1971) but can be distinguished from each other by their differing specificities for hapten. In addition, the cells mediating passively transferred delayed reactions show differences in sensitivity to the cytotoxic effects of a specific anti-thymus-derived lymphocyte (T-cell) serum, very small amounts of serum being adequate to kill the cells mediating contact sensitivity (Godfrey, 1976b).

The preceding paper (Godfrey, 1976a) demonstrated that the lymph node cells (LNC) of guinea-pigs immunized with 1-thiocyano-2,4-dinitrobenzene (DNTB) could be stimulated by reactive sensitizers and by hapten–protein conjugates to release migration inhibition (MIF) and macrophage agglutination (MAF) factors *in vitro* and that the release of MAF depended on the interaction of antigen with hapten-specific antigen receptors. Before further characterization of the antigen receptors on these sensitized cells could be undertaken, it would be necessary to separate them from the bulk of LNC, since the active cells form only 0·1–0·2 per cent of the cell population (McCluskey, Benacerraf and McCluskey, 1963; Turk and Oort, 1963). Affinity chromato-

Correspondence: Dr H. P. Godfrey, Department of Pathology, Health Sciences Center, State University of New York, Stony Brook, New York 11794, U.S.A.

graphy over derivatized polyacrylamide beads (Truffa-Bacchi and Wofsy, 1970) seemed an appropriate means to this end as it offered the dual advantages of direct demonstration of hapten-specific T cells in sensitized LNC populations and a very low non-specific cell loss in comparison with other chromatographic methods employing plastic beads or dextran (Wigzell and Mäkelä, 1970; Davie and Paul, 1970). The results of these studies indicate that affinity chromatography can be used to purify and concentrate hapten-specific cells mediating *in vitro* reactions which correlate with delayed hypersensitivity (DH).

MATERIALS AND METHODS

Animals

Male Hartley guinea-pigs, 350 ± 50 g at time of sensitization, were obtained from OLAC Northern, Red Hill Farm (Birmingham) or Statens Seruminstitute (Copenhagen, Denmark). They were housed four to a cage, fed guinea-pig pellets and their water supplemented with ascorbic acid.

Sensitization

Animals were sensitized once with 1 mg DNTB (K+K Laboratories, Plainview, New York) in 1 ml Freund's complete adjuvant (FCA) containing 1 mg heat-killed mixed strains of human *Mycobacterium tuberculosis* (Weybridge) and contact tested with the sensitizer up to three times (Godfrey and Baer, 1971). Animals taken 14 days after sensitization had not been skin tested; the majority of animals used had received three skin tests and showed threshold sensitivity to 0·4 or 2 nmoles DNTB by contact.

Preparation of chromatographic substrates

(A) Five grams of polyacrylamide beads Biogel P6, 16–20 Mesh (Bio-Rad Laboratories, Riverside, California) were swollen in 50 ml distilled water containing 150 mg bovine serum albumin (BSA) (Armour Pharmaceuticals) and a tracer amount of ^{125}I-labelled BSA with slow magnetic stirring for 45 min. 0·1 ml of 25 per cent glutaraldehyde (Electron Microscopy grade, Light) was added and stirring continued overnight at room temperature. The beads were washed extensively in distilled water by decantation. They were found to contain 30 μg BSA/g of beads. After equilibration with isotonic borate buffer, pH 9·0, the beads were reacted with 5 mg 1-fluoro-2,4-dinitrobenzene (DNFB) (Eastman Organic Chemicals, Rochester, New York) (2 per cent methanolic solution) per gram, and then washed extensively in phosphate-buffered saline, pH 7·2, containing 3 mM KCl (PBS) before use. This preparation is referred to as DNP–BSA (glutaraldehyde).

(B) The hydrazide of polyacrylamide was prepared by the method of Inman and Dintzis (1969) using 4 N hydrazine and incubating the beads at 62·5° for 1 h. After cooling to 25° the beads were washed to neutrality with distilled water. The beads were then treated in various ways. (1) The supernatant liquid was removed and the beads equilibrated with isotonic borate buffer, pH 9·0; either DNFB, or recrystallized 1-chloro-2,4,6-trinitrobenzene (TNCB, BDH, Poole) as 1 per cent methanolic solutions were slowly added dropwise with stirring at room temperature, to a final addition of 1 mg of chemical/g of beads. The beads began to colour after 5 min, the reaction being complete (by colour) at 30 min. Stirring was continued over 18 h. There was only slight colour in the supernate. The beads were extensively washed with PBS, pH 7·2, before use. These preparations are referred to as DNP–hydrazide and TNP–hydrazide respectively. (2) Polyacrylamide hydrazide beads were equilibrated with 0·25 N HCl and the acylazide derivative formed as described (Inman and Dintzis, 1969), at 4°. The beads were washed with cold water and trace labelled ^{125}I-labelled BSA added as a 2·5 per cent solution (6–8 mg BSA added/g of beads) in 0·33 M borate buffer. The pH was raised to and kept at 9·0 with 1 N NaOH over 30 min. Stirring at room temperature was continued for 45 min more and the beads left in pH 9 isotonic borate buffer overnight. The BSA acylazide preparation used in these studies had 4·3 mg BSA/g beads. They were washed extensively with PBS, pH 7·2, before any further use. DNP–BSA acylazide and TNP–BSA acylazide beads were prepared by reacting BSA acylazide beads with either DNTB or TNCB (1–2 mg/g of beads) as described above. (3) DNP–guinea-pig albumin (DNP–GPA) acylazide beads were prepared in a similar manner to DNP–BSA acylazide beads.

Preparation of lymph node cells (LNC) and technique of chromatography

LNC from sensitized and normal guinea-pigs were prepared by standard methods (Oppenheim, Wolstencroft and Gell, 1967). They were washed twice in tissue culture medium RPMI 1640 con-

taining HEPES (*N*-2-hydroxethylpiperazine-*N*′2-ethanesulphoric acid) pH 7·2, $NaHCO_3$ 0·85 per cent, glutamine, penicillin and streptomycin and 10 per cent foetal calf serum (1640–FCS) (Bio-Cult, Glasgow, Scotland) and resuspended to $1–2 \times 10^8$ cells/ml. Cell viability was measured by trypan blue exclusion. Four to 5 g of chromatography substrates were poured into sterile glass chromatography tubes (1 × 30 cm) with coarse glass sinters in the bottom and washed with 2000 ml PBS, pH 7·2, at 4°. Maximum flow rate was about 50 ml/min at 4 cm water pressure. After washing was complete, $1–2 \times 10^8$ normal or sensitive LNC were run into the column slowly, washed in with two 1-ml portions of PBS, pH 7·2, and immediately eluted from the column with PBS (flow rate: 16–20 ml/minute). A total of 200 ml of eluate was collected and the columns were then eluted with 1 ml of 100 mM DNP–glycine (chromatographically pure, BDH, Poole) in 200 mM phosphate buffer, pH 7·0, and the next 40 ml of eluate collected (flow rate: 16–20 ml/minute). The columns were washed with PBS until clear (approximately 1500 ml) and then washed with a further 1000 ml before re-use.

On those occasions when it was the aim to demonstrate hapten specificity of the responses, the cells to be chromatographed were taken up in 1640-FCS containing 1 mM DNP–glycine, pH 7·2. One millilitre of this suspension was applied to the column and washed in with two 1-ml portions of PBS with 1 mM DNP–glycine, pH 7·2. The cells were eluted at a flow rate of 20 ml/min with PBS containing 1 mM DNP–glycine and the first 50 ml of eluate collected. The columns were washed with 300 ml of PBS without DNP–glycine. Specific elution with 1 ml of 100 mM DNP–glycine was then carried out as described above and the specific eluate collected.

A volume of cells equal to that put on the column was diluted with PBS and labelled 'before' (B). The cells eluted by PBS without specific binding were labelled 'after' (A), while the cells specifically eluted with DNP–glycine were labelled 'eluted' (E). B, A and E cells were centrifuged at 1000 r.p.m. in a refrigerated centrifuge, washed twice in 1640–FCS, resuspended, counted and the viability determined before they were used in assays of DH or antigen binding.

Antigen-specific rosettes

DNP sheep red cells (DNP–SRBC) and control sheep red cells (SRBC) were prepared by the method of Layson and Sehon (1967). 250,000–500,000 LNC in 25 μl 1640–FCS were mixed with 0·2 per cent red cells in 100 μl 1640–FCS at 4°. They were incubated at 4° for 1 h, centrifuged at low speed and counted as wet smears (Coombs, Gurner, Janeway, Wilson, Gell and Kelus, 1970). The results are reported as rosette forming cells (RFC)/10^6 LNC. The hapten specificity of the DNP–RFC was determined by addition of DNP–glycine, pH 7·2, to a final concentration of 1 mM to the LNC–red cell mixture before incubation.

Indirect MIF and MAF assays

Washed B, A, and E cells were cultured at 4×10^6, 4×10^6 and 2 to $2{\cdot}5 \times 10^4$ cells/ml in 1640–FCS for 18 to 24 h at 37° in sterile plastic culture tubes (NUNC A/S, Roskilde, Denmark). Dilutions of B and A cells adjusted to the same cell density as the E cells were also cultured in the same manner. At the end of the culture period the supernatants were removed and immediately assayed for MIF and MAF activity as previously described (Godfrey, 1976a). The results of the MIF assay are presented as a mean relative migration index (per cent),

$$= \left[1 - \frac{\left(\dfrac{\text{Migration with antigen}}{\text{Migration without antigen}}\right)_N}{\left(\dfrac{\text{Migration with antigen}}{\text{Migration without antigen}}\right)_S} \right] \times 100,$$

where the subscripts S and N refer to results obtained with sensitive and normal cells respectively. The results of the MAF assays are presented as geometric mean relative titres $(\text{MAF titre})_S/(\text{MAF titre})_N$, where S and N again refer to the results from sensitive and normal cells. This method of presenting MAF titres was chosen when it was seen that the MAF titres obtained on testing normal cell suspensions were not significantly different from background values (Student's *t*-test).

Blast transformation assays

These were performed using 1×10^6 B or A cells cultured for 72 h in 1 ml medium in sterile plastic tubes and harvested as described (Oppenheim *et al.*, 1967). 0·5 μCi [^{3}H]thymidine (Amersham) was added to the cultures 16 h before harvesting. The results are presented as the mean ratios of d.p.m. in stimulated and control cultures of two to five experiments.

Assays for mitogenic factor were performed as described by Wolstencroft and Dumonde (1970).

Statistical treatment
All data were evaluated by the appropriate use of Student's *t*-test and analysis of variance.

RESULTS

Recovery and viability of LNC after affinity chromatography

There was little non-specific loss of either sensitive or normal LNC after chromatography over substituted polyacrylamides. An average of 100 per cent (±4 per cent, standard error) of the applied cells were eluted by PBS in thirty-six experiments using different substrates. The overwhelming majority (99·6–99·9 per cent) of the cells not specifically bound to the column were found to be in the first 40 ml of PBS; the next 80 ml contained 0·09–0·3 per cent of the cells finally recovered and a further 80 ml contained 0·02–0·09 per cent. The standard procedure of cellular chromatography was therefore to collect the first 40 ml of PBS eluate and to then wash the column with a further 250 ml PBS before attempting specific elution with DNP glycine. After specific elution, a further 0·02 to 0·5 per cent (0·143 ± 0·002 per cent, mean ± standard error) of the original number of cells were recovered. In several cases a greater number of cells were recovered from normal than from sensitive LNC populations.

Column chromatography over polyacrylamide did not remove non-viable cells from LNC and the viability of specifically eluted cells from these columns was essentially the same as that of the starting LNC, 82–85 per cent. While only about 90 per cent of the starting LNC or LNC eluted by PBS were morphologically small lymphocytes, virtually all the specifically eluted cells were.

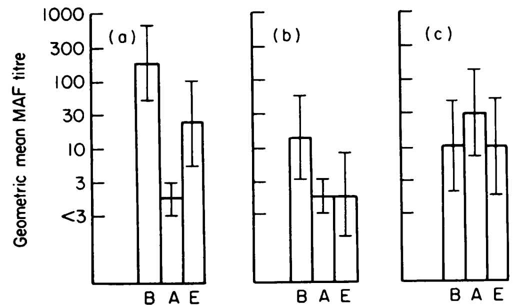

Figure 1. Geometric mean relative MAF (±s.e.m.) titres after incubation of DNP-sensitive and normal guinea-pig LNC with antigens before (B) and after (A) DNP–hydrazide column chromatography, and after specific elution with DNP-glycine (E). Relative MAF titre = $(\text{MAF titre})_S/(\text{MAF titre})_N$ where S and N refer to MAF titres obtained with sensitive and normal LNC respectively. See Materials and Methods section for details for assays and chromatography. Mean of six experiments. (a) DNFB; (b) DNP–BSA; (c) PPD.

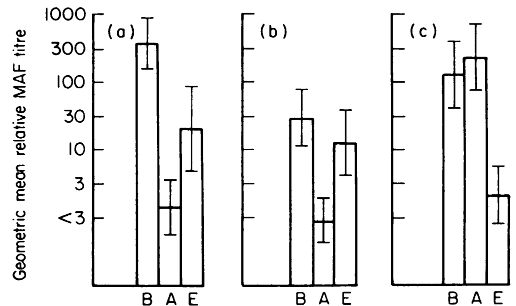

Figure 2. Effect of chromatography of DNP-sensitive and normal LNC over DNP–BSA–acylazide polyacrylamide on geometric mean (±s.e.m.) relative MAF titres. See Fig. 1 legend for details. Mean of ten experiments. (a) DNFB; (b) DNP–BSA; (c) PPD.

Cell chromatography over DNP-conjugated substrates
Cells mediating MAF release in response to DNFB were removed from LNC after chromatography of DNP-sensitive cells over all DNP-containing columns (Figs 1 and 2) while cells mediating MAF release in response to PPD were not. The reduction in MAF titres in response to DNP–BSA was statistically significant only after chromatography over columns containing DNP–BSA or DNP–GPA although a small reduction in MAF titre to DNP–BSA was seen after chromatography over DNP–hydrazide. Normal LNC did not show any statistically significant MAF response to antigen at any time.

Specifically eluted DNP-sensitive cells from DNP-hydrazide, DNP–BSA–acylazide and DNP–GPA-acylazide columns released MAF after exposure to DNFB but not after exposure to PPD. While specifically eluted cells from DNP–BSA or DNP–GPA containing columns responded to DNP–BSA, the relative MAF response of cells specifically eluted from DNP–hydrazide to this antigen was not statistically significant. Very few cells were specifically

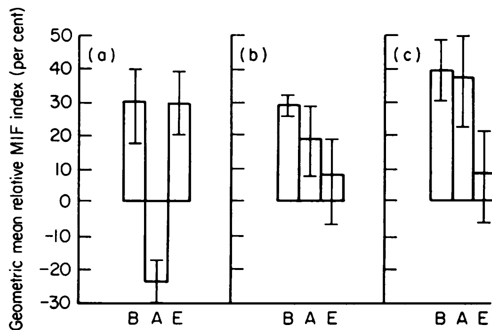

Figure 3. Effect of chromatography of DNP-sensitive and normal LNC over DNP–BSA–acylazide polyacrylamide on geometric mean (±s.e.m.) relative MIF. See Fig. 1 legend for details. See Materials and Methods section for details of assays. Mean of six experiments. (a) DNFB; (b) DNP–BSA; (c) PPD.

released from DNP-BSA (glutaraldehyde) columns and those released did not respond to any antigens. Dilutions of the original DNP sensitive LNC and of the PBS eluted LNC containing as few cells/ml as specifically eluted cells produced no MAF in response to antigen exposure.

Cells mediating MIF production in response to DNFB were removed by chromatography over DNP–BSA–acylazide, a procedure associated with a non-significant reduction in response to DNP–BSA and PPD (Fig. 3). The specifically eluted cells released MIF only after exposure to DNFB. Chromatography over DNP–GPA–acylazide did not remove cells mediating MIF in response to any of the three antigens.

Antigen-induced blast transformation to DNP–BSA, DNP–GPA or PPD was not affected by chromatography over DNP–BSA or DNP–GPA–acylazide columns (Table 1). Attempts to measure the ability of eluted cells to release mitogenic factor were not successful.

LNC preparations from both sensitive and normal guinea-pigs contained similar numbers of cells forming rosettes with DNP–SRBC and SRBC (Table 2). DNP rosettes of DNP–sensitive and normal LNC were hapten-specific, being significantly more inhibited in the presence of 1 mM DNP–glycine than SRBC rosettes. DNP rosettes of normal LNC were significantly more inhibited by this concentration of hapten than were those from sensitive LNC. DNP–RFC were depleted after chromatography over DNP–containing columns in contrast to SRBC–RFC whose numbers were unaffected by chromatography (Table 3). Specifically eluted cells from these columns did not contain increased concentrations of DNP–RFC, however.

Specificity of cell chromatography

The binding of cells releasing MAF in response to DNP was inhibited by 1 mM DNP–glycine (Fig. 4) as was the binding of DNP–RFC (Table 3). These observations indicate the presence of hapten-specific receptors for DNP on cells mediating MAF release in response to DNFB and to DNP–BSA,

Table 1. Effect of affinity chromatography on antigen-induced blast transformation

Column type	Donor of LNC	Response to indicated antigen					
		DNP–BSA		DNP–GPA		PPD	
		Before chromatography	After chromatography	Before chromatography	After chromatography	Before chromatography	After chromatography
DNP–BSA	Sensitized	3·36*	4·33*	3·06*	2·15*	11·7*	5·2*
	Normal	1·62	1·20	1·15	1·66	1·26	0·63
DNP–GPA	Sensitized	4·68*	4·81*	3·06*	2·32	18·1*	9·89*
TNP–BSA	Sensitized	1·78	0·99	2·91*	3·35*	4·23*	6·64*
	Normal	1·92	2·13	1·70	5·11	1·48	1·52

* Ratio of geometric mean net counts in antigen-stimulated LNC cultures to geometric mean net counts in unstimulated cultures (see Materials and Methods section for details). The ratios that are significantly greater than 1·0 ($P < 0{\cdot}05$) are marked with an asterisk. In no case was there a significant change in stimulation ratio after chromatography.

Table 2. Inhibition of DNP–SRBC and SRBC rosette formation in the presence of 1 mM DNP–glycine

Days after immunization	DNP–SRBC RFC/10^6 LNC		SRBC RFC/10^6 LNC	
	PBS	DNP–glycine	PBS	DNP–glycine
84	20·0	8·0	12·0	0·0
84	76·0	24·0	60·0	60·0
90	4·4	0·0	n.d.	n.d.
90	13·3	4·4	71·0	39·8
101	72·0	40·0	168	160
101	n.d.	n.d.	56·0	72·0
110	80·0	40·0	240	250
Normal	44·0	12·0	15·6	20·0
Normal	4·0	0·0	32·0	12·0
Normal	17·8	0·0	13·3	13·3
Normal	106·6	22·1	165	191

The Materials and Methods section gives details of assay. The inhibition of rosette formation with DNP–SRBC is significantly larger than that seen with SRBC whether LNC were from sensitized or normal donors ($P<0·05$ and $P<0·025$ respectively). Rosette formation of normal LNC with DNP–SRBC was significantly more inhibited by hapten than rosette formation of LNC from sensitized animals ($P<0·05$); rosette formation of sensitive and normal LNC with SRBC in the presence of hapten was not significantly different ($P<0·3$) from each other or from 0.

n.d. = Not determined.

Table 3. Effect of affinity chromatography on DNP- and SRBC–rosette-forming cells

Column type	Donors	Percentage change DNP–rosette-forming cells/10^6 LNC*	Percentage change SRBC rosette-forming cells/10^6 LNC
DNP–BSA–acylazide	Yes	−54±11†‡§	3±7†
	No	−40±9	−11±6
DNP–BSA–acylazide +1 mM DNP–glycine	Yes	26±13§	15±28
DNP–BSA (glutaraldehyde)	Yes	−75±16	21±8
DNP–hydrazide	Yes	−43±13	3±5
	No	−46±3	3±2
TNP–BSA–acylazide and TNP–hydrazide	Yes	−65±15	0·0
	No	−36±0·0	0·0
BSA–acylazide	Yes	8±8‡	n.d.
	No	25	n.d.

See Materials and Methods section for details of chromatography.

* Mean±s.e.

† Means are significantly different $P<0·001$.

‡ Means are significantly different $P<0·02$.

§ Means are significantly different $P<0·05$.

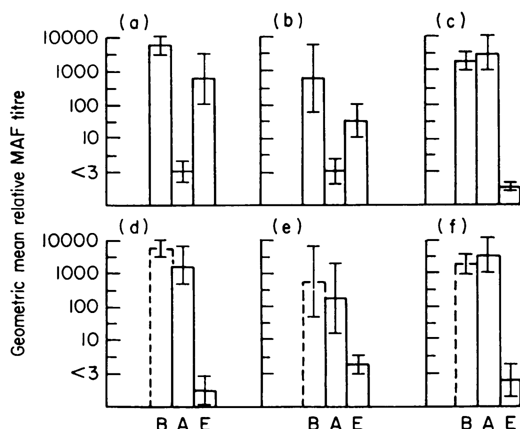

Figure 4. Inhibition of specific binding to DNP–BSA-acylazide polyacrylamide by free hapten. (a)–(c). Effect of column chromatography of DNP-sensitive and normal LNC on geometric mean ($\pm$s.e.m.) relative MAF titres in the absence of 1 mM DNP–glycine. (d)–(f) Effect of column chromatography on the same LNC in the presence of 1 mM DNP–glycine. See Fig. 1 legend for details. See Materials and Methods section for details of assays. Mean of six experiments. (a) and (d) DNFB; (b) and (e) DNP–BSA; (c) and (g) PPD.

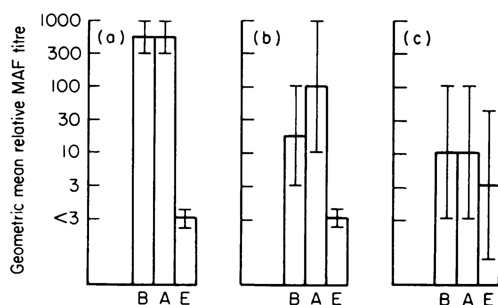

Figure 5. Effect of column chromatography of DNP-sensitive and normal LNC over TNP–hydrazide and TNP–BSA-acylazide polyacrylamide on geometric mean ($\pm$s.e.m.) relative MAF titres. See Fig. 1 legend for details. Mean of four experiments. (a) DNFB; (b) DNP–BSA; (c) PPD.

and on DNP–RFC. (DNP–glycine was present only during chromatography itself and was removed before any assays were performed.)

Chromatography of sensitive and normal LNC over TNP-containing substrates provided a further control of the specificity of cell binding (Fig. 5). In contrast to the results with DNP–containing substrates, TNP-modified polyacrylamides did not remove DNP–reactive cells mediating MAF release. However, TNP–containing columns were similar to DNP ones in that they could deplete DNP–RFC (Table 3) but not cells associated with antigen-induced blast transformation (Table 1). BSA–acylazide columns which contained neither DNP or TNP groups removed no antigen responsive cells confirming that the observed effects of affinity chromatography were dependent on the presence of DNP and TNP groups in the substrate (Table 3).

DISCUSSION

Antigen-mediated affinity chromatography has been successfully used to deplete T cells as well as B cells (bone marrow-derived lymphocytes) from lymphocyte populations (Davie and Paul, 1970; Rubin and Wigzell, 1973) as well as to isolate T cells (Rutishauser, D'Eustachio and Edelman, 1973), despite initial difficulties encountered with this technique (Wigzell and Mäkelä, 1970). Although we were not able to repeat the observation of Davie and Paul (1970) that cells mediating antigen induced blast transformation could be depleted by affinity chromatography, we have shown that the technique can be successfully used to separate DNP-sensitive cells mediating the release of MIF and MAF from those responding to PPD as well as from the bulk of LNC. Antigen-induced release of MIF and MAF has been shown to be one property of T cells (Yoshida, Sonozaki and Cohen, 1973; Godfrey, unpublished observations) as well as B cells (Yoshida *et al.*, 1973; Rocklin, MacDermott, Chess, Schlossman and David, 1974). However, since DNP–rosette-forming cells, a cell population likely to have immunoglobulin antigen receptors (Gell and Godfrey, 1974) and therefore likely to be B cells, were not found to be specifically eluted under the conditions of our experiments, it might be argued that the specifically eluted cell populations consist of MIF releasing T cells only. All DNP-sensitive cells releasing MAF are likely to be T cells since they were sensitive to treatment with a specific cytotoxic anti-guinea-pig T-cell serum (Godfrey, unpublished observations).

Binding of DNP-sensitive LNC was found to be inhibitable by free hapten indicating that the antigen receptors of these cells releasing MAF are hapten specific. This hapten specificity was not unexpected in view of the hapten specificity of the MAF response demonstrated in the preceding paper (Godfrey, 1976a). A somewhat more surprising finding in view of the differences in specificity between delayed-

onset sensitivities to DNTB and DNP–BSA *in vivo* (Godfrey, 1976a) was the observation that cells releasing MAF in response to DNP–BSA were not bound by TNP-containing polyacrylamides. Since the *in vivo* contact response in DNTB-sensitized guinea-pigs was highly specific for DNTB, one might predict the inability of a TNP column to bind cells responding to DNFB. Because the delayed-onset skin response to DNP–BSA was elicited fairly well also by TNP–BSA *in vivo* it might have been supposed that DNP–BSA-responsive cells would be bound to a TNP column. Our observations indicate that the antigen receptors of DNP–BSA-responsive cells are more specific for DNP than the results of skin testing would suggest.

In spite of the overall similarity we observed in the specificity of the antigen receptors of MAF releasing LNC in response to the two DNP antigens, the possibility still exists that the antigen receptors of cells responding to DNP–BSA differ from those responding to DNFB. This is suggested by the observation that affinity chromatography over DNP–BSA columns, a procedure which depleted DNFB–responsive cells, did not deplete the cells releasing MIF or undergoing blast transformation in response to DNP–BSA.

Hapten-specific DNP–RFC were not eluted under conditions which eluted hapten-specific cells releasing lymphokines. The specificity of the antigen receptors of these RFC also differed from those involved in lymphokine release in that they were depleted to the same degree by TNP– and DNP–containing substrates and were readily inhibited by anti-immunoglobulin (Rolley and Marchalonis, 1972; Gell and Godfrey, 1974). These observations suggest that these cells are not associated with delayed-onset hypersensitivities, especially when taken in conjunction with the known cross-reactivity of DNP and TNP antibodies (Steiner and Eisen, 1967). They could be associated with the low levels of anti-DNP antibodies seen in DNTB immunized animals. It has previously been reported that the specificity of antigen receptors on RFC was the same as that of serum antibody and different from that involved in another cellular immune response, blast transformation (Davie, Rosenthal and Paul, 1971). Hence while RFC usually have immunoglobulin-like antigen receptors, other cells involved in cell-mediated responses may not. The relation between immunoglobulin receptors and DH remains an open question.

ACKNOWLEDGMENTS

We wish to thank Miss J. Lowe, Hazel Stokes and Hanne N. Nors for their valuable technical assistance. This work was begun while H. P. Godfrey was a Moseley Travelling Fellow of Harvard University and had been supported by grants from the Anna Fuller Fund, the British and Danish Medical Research Councils, and Mary Rosenkjaers Legat.

REFERENCES

Coombs R.R.A., Gurner B.W., Janeway C.A., Jr, Wilson A.B., Gell P.G.H. & Kelus A. (1970) Immunoglobulin determinants on the lymphocytes of normal rabbits. I. Demonstration by the mixed antiglobulin reaction of determinants recognized by anti-γ, anti-μ, anti-Fab and anti-allogype sera, anti-As4 and anti-As6. *Immunology*, **18**, 417.

Davie J.M. & Paul W.E. (1970) Receptors on immunocompetent cells I. Receptor specificity of cells participating in a cellular immune response. *Cell. Immunol.* **1**, 404.

Davie J.M., Rosenthal A.S. & Paul W.E. (1971) Receptors on immunocompetent cells. III. Specificity and nature of receptors on dinitrophenylated guinea-pig albumin—^{125}I-binding cells of immunized guinea-pigs. *J. exp. Med.* **134**, 517.

Dvorak H.F., Simpson B.A., Bast R.C., Jr & Leskowitz S. (1971) Cutaneous basophil hypersensitivity III. Participation of the basophil in hypersensitivity to antigen–antibody complexes, delayed hypersensitivity, and contact allergy. Passive transfer. *J. Immunol.* **107**, 138.

Gell P.G.H. & Benacerraf B. (1961) Studies on hypersensitivity. IV. The relationship between contact and delayed sensitivity: a study on the specificity of cellular immune reaction. *J. exp. Med.* **113**, 571.

Gell P.G.H. & Godfrey H.P. (1974) Studies on cell separations in delayed hypersensitivity. *Monogr. Allergy*, **8**, 89.

Godfrey H.P. (1976a) Hapten-specific responses to contact sensitizers: use of fluorodinitrobenzene to elicit migration inhibition and macrophage agglutination factors from lymph node cells of contact sensitive guinea pigs. *Immunology*, **30**, 685.

Godfrey H.P. (1976b) Differences in sensitivity to a cytotoxic anti-thymus-derived lymphocyte serum of cells mediating delayed-onset reactions in guinea pigs to hapten–protein conjugates and contactants. *Cell. Immunol.* (In press.)

Godfrey H.P. & Baer H. (1971) The effect of physical and chemical properties of the sensitizing substance on the induction and elicitation of delayed contact sensitivity. *J. Immunol.* **106**, 431.

Inman J.K. & Dintzis H.N. (1969) The sensitization of cross linked polyacrylamide beads. *Biochemistry*, **8**, 4074.

Layson M.N. & Sehon A.H. (1967) Detection of cells producing antibodies against the dinitrophenyl group. *Canad. J. Biochem.* **45**, 1773.

McCluskey R.T., Benacerraf B. & McCluskey J.W. (1963) Studies on the specificity of the cellular infiltrate in delayed hypersensitivity reactions. *J. Immunol.* **90,** 466.

Oppenheim J.J., Wolstencroft R.A. & Gell P.G.H. (1967) Delayed hypersensitivity in the guinea pig to a protein-hapten conjugate and its relationship to *in vitro* transformation of lymph node, spleen, thymus and peripheral blood lymphocytes. *Immunology,* **12,** 89.

Rocklin R.E., MacDermott R.P., Chess L., Schlossman S.F. & David J.R. (1974) Studies on mediator production by highly purified human T and B lymphocytes. *J. exp. Med.* **140,** 1303.

Rolley R.T. & Marchalonis J.J. (1972) Antigen binding lymphocytes in the early development of contact sensitization. *Transplantation,* **14,** 118.

Rubin B. & Wigzell H. (1973) Hapten-reactive helper lymphocytes. *Nature (Lond.),* **242,** 467.

Rutishauser U., D'Eustachio P. & Edelman G.M. (1973) Immunological function of lymphocytes fractionated with antigen-derivatized fibers. *Proc. nat. Acad. Sci. (Wash.),* **70,** 3894.

Steiner L.A. & Eisen H.N. (1967) The relative affinity of antibodies synthesized in the secondary response. *J. exp. Med.* **126,** 1185.

Truffa-Bacchi P. & Wofsy L. (1970) Specific separation of cells on affinity columns. *Proc. nat. Acad. Sci. (Wash.),* **66,** 685.

Turk J.L. & Oort J. (1963) A histological study of the early stages of the development of the tuberculin reaction after passive transfer of cells labelled with ^{3}H-thymidine. *Immunology,* **6,** 140.

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

Wolstencroft R.A. & Dumonde D.C. (1970) *In vitro* studies of cell-mediated immunity. I. Induction of lymphocyte transformation by a soluble 'mitogenic' factor derived from interaction of sensitized guinea-pig lymphoid cells with specific antigen. *Immunology,* **18,** 599.

Yoshida T., Sonozaki H. & Cohen S. (1973) The production of migration inhibition factor by B and T cells of the guinea pig. *J. exp. Med.* **138,** 784.