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Immunogenic cells in the regional lymph nodes after painting with the contact sensitizers picryl chloride and oxazolone: evidence for the presence of IgM antibody on their surface

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Summary. The lymph node cells of mice painted with contact sensitizing agents immunize recipient mice when injected into their footpads. In practice 2×10^6 nylon wool purified T cells are used from mice painted with picryl chloride or oxazolone (4-ethoxymethylene-2-phenyloxazolone). The ability of cells taken 4 days after painting to immunize other mice was abolished by treatment with rabbit complement but cells taken at 1 day were unaffected. This effect of rabbit complement was due to IgM anti-hapten antibody on the surface of antigen-presenting cells. The antibody could be eluted from the cells with appropriate picryl or oxazolone-ε-aminocaproic acid. It adhered to insolubilized anti-IgM and behaved like IgM on gel filtration. To confirm the role of this antibody, mice were rendered unresponsive with picrylated pneumococcal polysaccharide type III before being painted. This abolishes antibody production but leaves contact sensitivity intact. The lymph node cells of animals treated in this way were unaffected by rabbit complement and this suggested that antibody was required for this phenomenon. Moreover although lymph nodes normally lose the ability to immunize at day 6 after painting, the lymph node cells of unresponsive mice, which fail to make antibody immunize other mice up to day 8 after painting. This effect of unresponsiveness is reversed by the injection of serum taken 8 days after painting. It was concluded that IgM antibody which appears on the surface of lymph nodes 4 days after painting depresses their ability to immunize other mice.

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INTRODUCTION

It is known that various lymphoid cells conjugated with hapten *in vitro* immunize other animals when injected systemically and give rise to contact sensitivity without the use of adjuvant (Baumgarten & Geczy, 1970; Greene, Sugimoto & Benacerraf, 1978). This system has been used to study the cells involved in the induction of contact sensitivity. However, it is more physiological to use cells haptenized *in vivo* by painting the skin with contact sensitizer and to harvest the regional lymph node cells 1 or more days later (Asherson & Mayhew, 1976; Asherson, Zembala & Mayhew, 1977). There is good evidence that these cells behave like antigen-presenting cells when injected into the footpad of normal recipients and cause an active immune response. For instance, they induce contact sensitivity even when irradiated at 2000 rad *in vitro* and the recipients only become immune after a latency of 3–4 days. The ability of lymph node cells to

immunize other mice declines by day 4 after skin painting and is virtually absent by day 7 and specific anti-hapten antibodies can be found in the serum shortly before this time (Thomas, Watkins, Wood & Asherson, 1978).

This paper investigates the possible role of anti-hapten antibody in the decline of the ability of lymph node cells to immunize other mice. It was prompted by the finding that lymph node cells taken 4 days after immunization fail to immunize other mice after incubation with rabbit complement. This paper shows that cell-associated immune complexes are present in the regional lymph nodes 4–6 days after the skin is painted and may represent one level of control of the persistence of antigen in a form which is immunogenic.

MATERIALS AND METHODS

Mice

Eight to twelve week old CBA mice bred at the Clinical Research Centre were used. In any one experiment only one sex was used. There were four to five mice in each experimental group.

Immunization with picryl chloride and oxazolone

Mice were shaved on the thorax and abdomen and painted with 0·15 ml 5% ethanolic picryl chloride (BDH, Poole) or 3% oxazolone (4-ethoxymethylene-2-phenyloxazolone, Ox, BDH) on the skin and forepaws.

Induction of contact sensitivity by footpad injection

Donors were painted with picryl chloride (pic) or oxazolone (ox). The regional shoulder girdle and inguinal lymph nodes were removed 24 hr or 4 days later, dissociated in the standard medium (Eagle's minimal essential medium/Dulbecco's phosphate-buffered saline, 1:1) and washed twice. The cell population was then enriched in T lymphocytes by nylon wool filtration. Cells were irradiated at 2000 rad *in vitro* from a cobalt source and viability was assessed by trypan blue dye exclusion. Cells (2×10^6) in 0·1 ml were injected into each mouse, i.e. each hind footpad received 0·05 ml.

Detection of contact sensitivity

Five days after skin painting or after injection of cells into the footpads, the mice were challenged by painting both sides of both ears with 1% picryl chloride or oxazolone in olive oil and the increment of ear thickness measured at 24 hr with an engineer's micrometer and expressed in units of 10^{-3} cm $\pm$ SD. Student's t test was used to compare the differences between the means. Significant and highly significant refer to $P < 0{\cdot}05$ and $P < 0{\cdot}02$ respectively.

Treatment with complement

Normal rabbit serum selected for low cytotoxicity was stored at $-70°$. Rabbit C diluted 1/5 and normal mouse serum diluted 1/3 were used as a source of complement. Cells (10^7) were incubated with diluted rabbit or mouse serum (1 ml) at 37° for 1 hr. Cells were then washed twice and viability assessed by trypan blue.

Antibody titration and Jerne plaque assay

The method for detecting haemagglutinating antibody against picrylated cells and the slide modification of the method of Cunningham & Szenberg (1968) for detecting plaque forming cells are described in Thomas *et al.* (1978).

Production of unresponsiveness with picrylated pneumococcal polysaccharide

Picryl-ε-N-lysine was conjugated to type III pneumococcal polysaccharide (Mitchell, Humphrey & Williamson, 1972). A dose of 400 μg was injected intravenously 7 days before painting with picryl chloride.

Elution of antibody from cells and immunochemical characterization

The preparation of haptenized EACA is described in Asherson, Zembala & Noworolski (1978). Lymph node T cells taken 4 days after painting were treated at 4° with oxazolone or picryl-ε-aminocaproic acid (ox or pic-EACA) using 10^8 cells in 2 ml, containing 4 mg of neutralized haptenized EACA. After 1 hr the cells were spun down and the supernatant collected and dialysed against phosphate-buffered saline (PBS). Solid phase immunoadsorbent was prepared by conjugating rabbit affinity purified anti-mouse IgM absorbed with insolubilized mouse Ig to cyanogen bromide activated Sepharose 4B. The anti-mouse IgM beads were packed into a 5 ml syringe and the eluate from the cells (concentrated ten-fold) was applied in a volume of 2 ml and kept overnight at 4°. The column was then washed with 20 ml of PBS and the effluent collected. The IgM antibody was eluted with 0·1 M glycine buffer pH 2·8 (20 ml) and neutralized immediately with 1 M

NaOH. The effluent and eluate were then concentrated to 2 ml by Amicon membrane filtration.

Gel filtration was undertaken on Ultragel Whatman AcA34 and the fractions corresponding to IgM were pooled and concentrated back to 2 ml.

RESULTS

Effect of complement on the ability of lymph node cells from painted mice to induce contact sensitivity in normal recipients

When mice are painted with contact sensitizer they develop contact sensitivity and IgM, IgG and IgE antibodies. Moreover their lymph node cells act as antigen-presenting cells and induce contact sensitivity when injected into the footpads of normal recipients. This suggested that antigen occurred on the surface of the lymph node cells and it seemed possible that these cells might be coated with complement fixing anti-hapten antibody 4 days after immunization. To investigate this possibility the effect of rabbit complement on the ability of lymph node cells to immunize other mice was studied. Lymph node T cells were prepared from mice painted 1 or 4 days previously with oxazolone or picryl chloride. They were then incubated with rabbit or mouse complement, washed and 2×10^6 cells injected into normal recipients. The recipients were challenged on the ears 5 days later to assess contact sensitivity. Figure 1 (line two) shows that immunization by cells taken 1 day after painting was not depressed by treatment with rabbit complement. However, '4 day cells' entirely lost their ability to induce contact sensitivity (line four). Mouse complement had no effect.

Presence of anti-hapten antibody on the surface of lymph node cells 4 days after painting

The following experiment investigated the possibility that the effect of rabbit complement in blocking the immunizing ability by 4 day cells was due to antibody–antigen complexes on the surface of the lymphocytes. Advantage was taken of the fact that specific low molecular weight hapten dissociates immune complexes. Lymph node T cells from mice immunized with oxazolone or picryl chloride 4 days previously were treated with ox- or pic-ε-aminocaproic acid at 4° *in vitro*. After washing the cells were incubated in rabbit complement at 37° and then injected into recipients to test their ability to induce contact sensitivity. Figure 2 confirms that rabbit complement virtually abolished the ability of 4 day lymph node cells to induce contact sensitivity (lines two and nine) and that this effect was prevented when the cells were preincubated with specific low molecular weight hapten. In particular pic-EACA blocked the effect of rabbit complement on immunization by picryl cells (line four) while ox-EACA

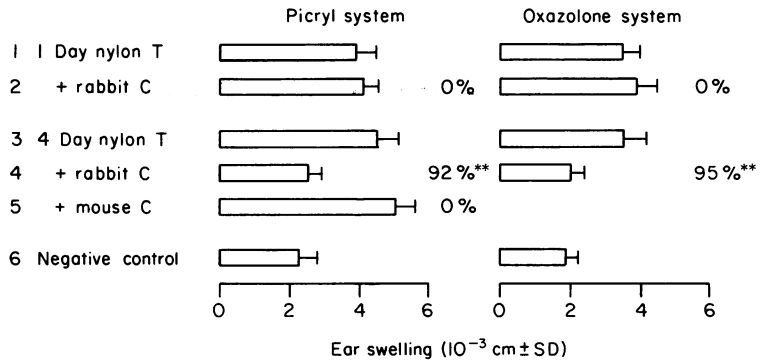

Figure 1. The effect of rabbit and mouse complement on the induction of contact sensitivity by cells from mice painted with picryl chloride or oxazolone. Nylon wool T cells (10^7/ml) from mice painted with picryl chloride or oxazolone were incubated under control conditions with rabbit C (1/5) or mouse C (1/3) for 1 hr. They were then washed and 2×10^6 injected into the hind footpads of groups of five mice. Contact sensitivity was assessed 5 days later and measured by the ear swelling at 24 hr in units of 10^{-3} cm ± SD. The negative control refers to mice which received no cells. The figures show the percentage inhibition of contact sensitivity (ear swelling) taking the difference between untreated cells and the negative control as 100%. ** Highly significant reduction of contact sensitivity.

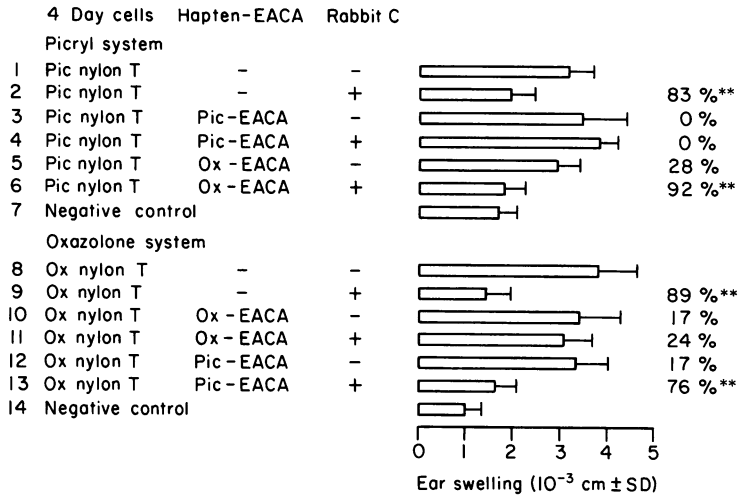

Figure 2. Rabbit complement inhibits the induction of contact sensitivity by cells taken 4 days after immunization with picryl chloride or oxazolone: inhibition of the effect of rabbit complement by treatment with the corresponding picryl or oxazolonyl-ε-aminocaproic acid. Nylon wool T cells from mice painted with picryl chloride or oxazolone were treated at 4° with picryl or oxazolonyl-ε-aminocaproic acid and incubated with rabbit complement. The cells were then injected into the footpads of groups of five mice and contact sensitivity assessed 5 days later. The figures show the percentage inhibition of contact sensitivity taking the difference between the untreated cells (lines one and eight) and the negative control (lines seven and fourteen) as 100%. ** Highly significant reduction of contact sensitivity.

had no effect (line six). The lower part of Fig. 2 shows similar results in the oxazolone system (lines eleven and thirteen).

This experiment suggested that low molecular weight hapten removed antibody from the surface of the lymph node cells. The following experiment shows that antibody appears in the eluate of cells treated with haptenized EACA. Use was made of the fact that lymph node cells taken 1 day after painting were unaffected by rabbit complement presumably because they were not coated with anti-hapten antibody (Fig. 3, lines one and two). However, '1 day cells' treated with the specific eluate from 4 day cells and then incubated with rabbit complement lost their ability to induce contact sensitivity (lines three and four). Taken together these results show that antibody is present on the surface of 4 day immune cells and can be removed by low molecular weight hapten.

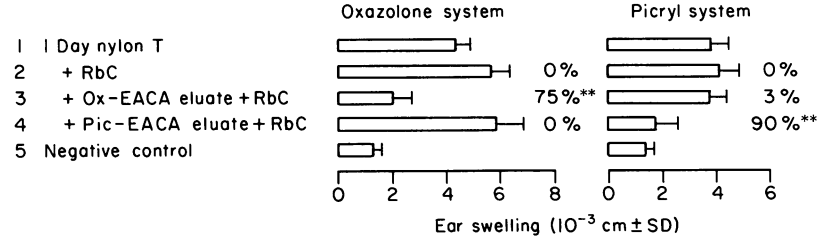

Figure 3. Cells taken 1 day after immunization with picryl chloride or oxazolone induce contact sensitivity when injected into other mice: the effect of treatment with eluates of cells taken 4 days after immunization followed by rabbit complement. Eluates were prepared from nylon wool T, '4 day' immune cells by treatment with picryl or oxazolonyl EACA. Cells taken 1 day after immunization were treated with the eluates (presumptive antibody) at 4° followed by rabbit C at 37°. The cells were then injected into groups of normal mice and contact sensitivity assessed 5 days later.

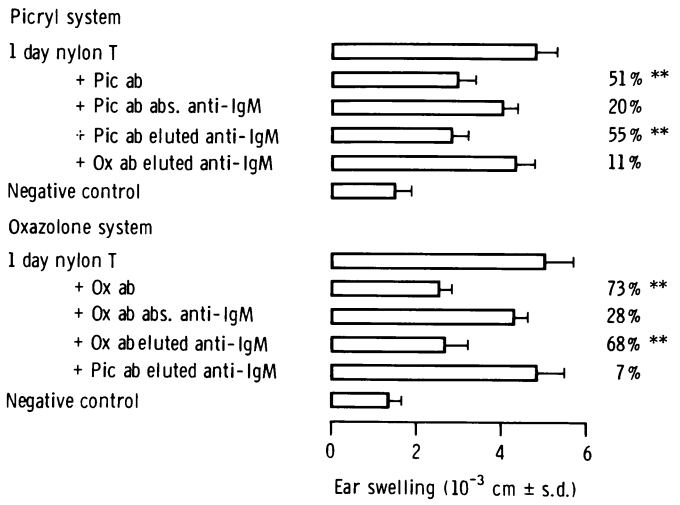

Figure 4. Absorption with insolubilized anti-IgM of specific eluates of cells taken 4 days after immunization with picryl chloride or oxazolone. Eluates were prepared from nylon wool T, 4 day immune cells by treatment with the corresponding picryl or oxazolonyl-ε-aminocaproic acid and are referred to as Pic and Ox antibody (ab). Some of the antibody was absorbed with insolubilized anti-IgM and antibody recovered by elution under acid conditions. The initial antibody, the absorbed antibody and the eluted antibody were tested for their ability to inhibit the induction of contact sensitivity by cells taken 1 day after immunization. See legend to Fig. 3. As a control in the picryl system, antibody against oxazolone was absorbed and eluted from anti-IgM to show that the elution process did not introduce non-specific toxic material.

Characterization of the antibody eluted from 4 day immune cells

The possibility that the specific factor eluted from 4 day cells was an IgM antibody was investigated by absorption and elution of the antibody from anti-IgM beads and by gel filtration. The following experiments show that the antibody eluted from 4 day cells was IgM.

In the first experiment antibody eluted from 4 day picryl immune cells with pic-EACA was absorbed by insolubilized anti-IgM antibody. The absorbed antibody was then eluted and the preparations tested for their ability to sensitize 1 day cells to the action of rabbit complement. Figure 4 shows that absorption significantly reduced the effect of antibody while the eluate from the IgM beads was active. The lower part of Fig. 4 shows similar results in the oxazolone system. The activity of the eluate from the anti-IgM column was specific as the anti-oxazolone factor after absorption and elution from anti-IgM beads did not inhibit 1 day picryl immune cells and *vice versa*. Figure 5 shows that the inhibitory activity behaved like IgM on gel filtration. It was concluded that 4 days after immunization lymph node cells were coated with IgM anti-hapten antibody.

Effect of inhibiting the anti-hapten response on the ability of lymph node cells to immunize other mice

The previous experiment showed that rabbit complement prevented 4 day cells from immunizing other mice and that this effect was due to IgM anti-hapten antibody–antigen complexes. However, as mouse complement had no effect it was not clear whether these antibody–antigen complexes had any action *in vivo*. To confirm the role of antibody *in vivo* the effect of suppressing the anti-hapten antibody response was studied. In the first experiment mice were rendered unresponsive by the injection of picrylated pneumococcal polysaccharide. This had no effect on contact sensitivity but inhibited the antibody and Jerne plaque response (Fig. 6). In keeping with this, the lymph node cells of mice rendered unresponsive and then painted were not significantly affected by rabbit complement. In contrast rabbit complement completely abolished the ability of lymph node cells from painted mice, which had not been pretreated, to immunize other mice (Fig. 7).

The following experiment compared the ability of lymph node cells from mice painted with picryl chloride and from mice made unresponsive with picrylated pneumococcal polysaccharide to immunize

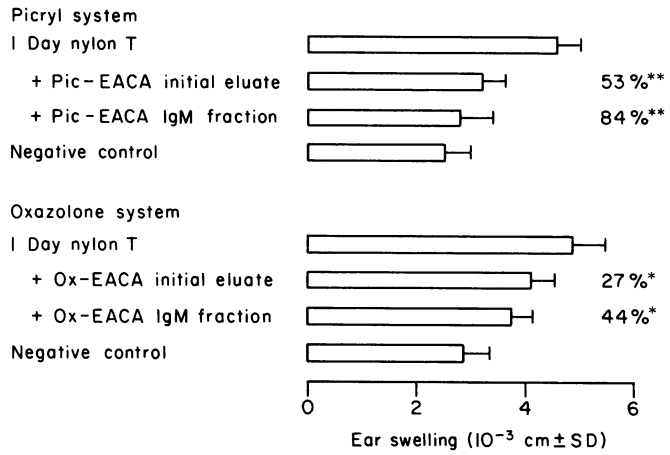

Figure 5. Gel filtration of specific eluates of cells taken 4 days after immunization with picryl chloride or oxazolone. Eluates were prepared from nylon wool T, cells (taken 4 days after immunization with picryl chloride or oxazolone) by treatment with the corresponding haptenized ε-aminocaproic acid. The eluates were concentrated ten-fold and 2 ml applied to an Ultragel column. The IgM fractions were pooled, concentrated back to 2 ml and tested for their ability, together with rabbit complement, to inhibit the induction of contact sensitivity by '1 day' immune cells.

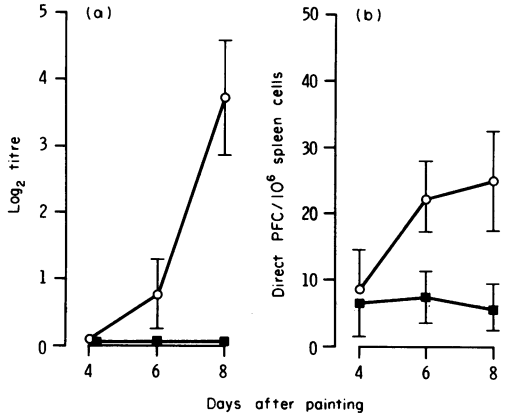

Figure 6. Effect of pretreatment of mice with picryl-pneumococcal polysaccharide type III on the antibody and contact sensitivity response to painting with picryl chloride. Mice were pretreated with picryl pneumococcal polysaccharide type III (400 μg intravenously). Seven days later the mice were painted with picryl chloride. (a) The haemagglutination titre in $\log_2$ units (2^1 is equivalent to an initial dilution of 1/2). The antibody was IgM as judged by treatment with 2-mercaptoethanol. Antibody response in normal mice (O——O); the failure of antibody response in the mice pretreated with picryl-polysaccharide (■——■). (b) Similar results using the Jerne plaque technique. Five days after immunization normal mice showed contact sensitivity of 5·3±0·55 as compared with a negative control of 2·2±0·50. Pretreatment with picryl-polysaccharide had no effect (5·1±0·74).

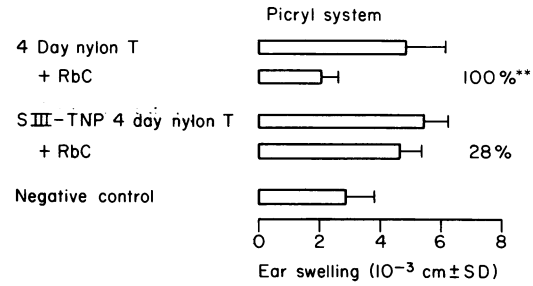

Figure 7. Rabbit complement prevents immunization by cells taken 4 days after immunization with picryl chloride: absence of the phenomenon in mice in which the antibody response was inhibited by pretreatment with picryl pneumococcal polysaccharide type III. Mice were pretreated with picryl (trinitrophenyl) pneumococcal polysaccharide type III (SIII-TNP) or left untreated. Seven days later they were immunized with picryl chloride and nylon wool T lymph node cells prepared 4 days later. The figure shows that rabbit C blocked the ability of 4 day immune cells from control mice to induce contact sensitivity but had no significant effect on the cells from mice pretreated with the picryl polysaccharide.

other mice. The open circles in Fig. 8 show that lymph node cells of normal painted mice immunized other mice when taken on day 4 but not when taken on day 6 or 8. In contrast, the lymph node cells of mice rendered unresponsive and then painted, immunized other mice when taken on days 4, 6 and 8 but had no effect on day 10. To confirm that this prolonged ability to immunize was due to the absence of antibody, the unresponsive mice were injected with 8 day immune serum on the day of immunization and 2 days later. This abolished the ability of both 4 and 6 day cells to immunize (Fig. 8). It was concluded that circulating antibody was important in limiting the persistence of antigen in a form able to induce contact sensitivity.

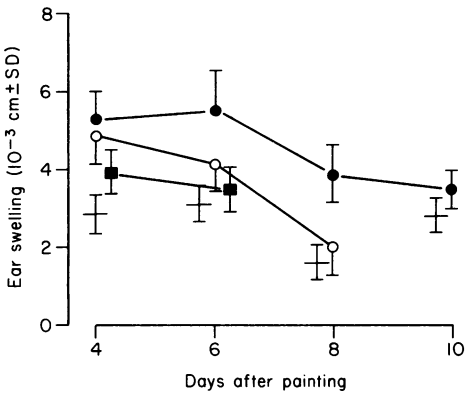

Figure 8. Ability of lymph node cells taken at various times after painting with picryl chloride to induce contact sensitivity in normal recipients: the effect of pretreatment with picryl pneumococcal polysaccharide and the reversal of this effect by immune serum. Mice were pretreated with picryl polysaccharide or left untreated. Seven days later they were immunized by painting with picryl chloride. Four, 6 and 8 days later, nylon T cells were prepared and tested for their ability to induce contact sensitivity. (○——○) The cells from mice which were not pretreated induced contact sensitivity when taken at 4 days, but were inactive at 6 and 8 days when compared with the negative control shown as short horizontal lines (——). In contrast (●——●) the cells from mice pretreated with picryl-polysaccharide and then immunized induced contact sensitivity in normal mice when taken 4, 6 or 8 days after painting but were inactive by day 10. (■——■) Cells from mice pretreated with picryl-polysaccharide and then painted and injected with immune serum (0·5 ml) on the day of painting and 2 days later. The serum was obtained from mice 8 days after a single paint with picryl-chloride. These cells failed to immunize whether taken on day 4 or day 6.

DISCUSSION

It is known that lymph node cells taken 1 or 4 days after painting the skin with a contact sensitizer behave like antigen-presenting cells and are able to immunize normal recipients. See Asherson, Zembala, Thomas & Perera (1980). This ability to immunize disappears by day 7 after painting which is shortly after the time of the appearance of anti-hapten antibody in the serum. This time relationship raised the possibility that antibody might limit the persistence of antigen in the lymph node in an immunogenic form. The basic observation was that rabbit complement destroyed the ability of 4 day but not of 1 day cells to immunize. This suggested that complement fixing, anti-hapten antibody–antigen complexes occurred on the surface of lymphoid cells 4 days but not 1 day after immunization.

Direct evidence for antibody on the cell surface was obtained by elution with low molecular weight hapten (picryl and oxazolone-ε-aminocaproic acid). Treatment of 4 day immune cells with the specific low molecular weight hapten abolished the effect of rabbit complement, presumably by removing anti-hapten antibody. In fact this antibody could be recovered in the eluate and rendered cells taken 1 day after immunization susceptible to the action of rabbit complement. The IgM nature of the antibody was shown by absorption with anti-IgM serum and gel filtration. The finding of immune complexes on the surface of lymph node cells is in keeping with the evidence of Kontiainen & Mitchison (1975) that antigen may persist on the surface of T cells in the form of immune complexes. The antibody or complement may in fact enable these cells to interact with cells with Fc and complement receptors. In the present experiment the antigen-bearing cell is probably a T cell as it does not adhere to nylon wool and previous experiments have shown that the ability of lymph node cells to induce contact sensitivity is partially or completely blocked by anti-Thy-1 serum and complement (Thomas, Edwards, Watkins & Asherson, 1980).

Until recently it was considered that only macrophages and related cells such as the Langerhans cell presented antigen. However, there is now evidence that B cell–B cell lymphoma hybridomas can present antigen (Glimcher, Hamano, Asofsky, Heber-Katz, Hedrick, Schwartz & Paul, 1982). In the present system lymph node cells from mice only immunize recipients when the H-2 of the injected cell is the same as that of the recipient and this is not due to an

allogeneic effect (Asherson, Mayhew & Perera, 1979). This implies that any re-presentation by the recipient's macrophages must keep together the H-2, and the oxazalone hapten of the injected cell. An alternative view is that direct presentation of antigen by T cells may occur. The issue is unresolved.

In fact other authors have shown that antigen-presenting cells are sensitive to anti-hapten antibody and complement (Thomas & Shevach, 1978) and that IgM antibody lyses nucleated cells with foreign (viral) antigens on their surface (Betts & Schmidt, 1981). However, there are few reports of IgM antibodies causing immune enhancement (Fuller & Winn, 1973; Rubinstein, Decary & Streum, 1974).

The fact that immune cells lose their ability to immunize other mice when treated with rabbit (but not mouse) complement *in vitro* raised the possibility whether the IgM antibody might be irrelevant to events *in vivo*; it seemed important to investigate whether serum antibody was active *in vivo*. For this reason mice were pretreated with picrylated pneumococcal polysaccharide and then painted. This pretreatment inhibited antibody production but not contact sensitivity. Experiment showed that the 4 day lymph node cells from these mice were unaffected by rabbit complement *in vitro* presumably because of the absence of antibody.

The cells from the unresponsive painted mice showed a second interesting property. The ability of lymph node cells to immunize usually disappears by 7 days after painting; in contrast the lymph node cells of unresponsive mice are able to immunize up to 8 days after painting. This prolonged ability to immunize disappeared when the unresponsive painted mice were injected with immune anti-picryl serum taken from mice 8 days after painting with picryl chloride. This implied that antibody *in vivo* was able to limit the persistence of antigen in the regional lymph nodes in a form able to immunize.

It is not clear how IgM antibody limits the ability of lymph node cells to immunize other mice. It is unlikely to be due to a direct lytic effect of mouse complement as this has no effect *in vitro* probably because it is not readily activated by IgM complexes (Grant, 1976) and this may be especially important when the complexes are formed at low antigen density (Borsos, Chapuis & Langone, 1981). However the antibody may mask antigen, or cause modulation or phagocytosis by macrophages or other Fc receptor positive cells. In fact, antibody is not the only antigen-specific factor which may influence the presentation of antigen. Ptak & Gershon (1982) have shown that picrylated lymphoid cells lose their ability to immunize other mice when incubated in T suppressor factor. This is not due to a simple masking effect, as doubly haptenized cells lose their ability to immunize when incubated in TsF specific for only one of the haptens. In any event the antibody is probably not responsible for the decline of contact sensitivity with time after immunization, as distinct from the decline in the ability to induce contact sensitivity in other mice. This is indicated by the experiments of Maguire, Faris & Weidanz (1979) in which contact sensitivity showed a normal time course in mice unable to make antibody.

The present findings have an interesting application in the study of T helper cells which augment contact sensitivity. These have been described in viral and protein antigen systems (Tucker & Bretscher, 1982). In contact sensitivity, T helper cells can be studied using 4 day immune cells treated with rabbit complement. The cells lose their ability to immunize other mice but provide T cell help and make T helper factor which enables small numbers of lightly picrylated spleen cells (10^6 cells picrylated with ImM picrysulphonic acid for 10 min) to induce contact sensitivity. The characteristics of these T helper cells and factors will be described in a subsequent paper.

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REFERENCES

ASHERSON G.L. & MAYHEW B. (1976) Induction of cell-mediated immunity in the mouse: circumstantial evidence for highly immunogenic antigen in the regional lymph nodes following skin painting with contact sensitizing agents. *Israel J. Med. Sci.* **12,** 454.

ASHERSON G.L., MAYHEW B. & PERERA M.A.C.C. (1979) The production of contact sensitivity by the injection into the footpads of recipients of the lymph node cells from mice 1 day after painting their skin with contact sensitizing agent: requirement for matching at the major histocompatibility complex between donor and recipient mice. *Immunology*, **37,** 241.

ASHERSON G.L., ZEMBALA M. & MAYHEW B. (1977) Analysis

of the induction phase of contact sensitivity by footpad transfer of regional lymph node cells: macrophages and radioresistant T cells induce immunity. *Immunology*, **32,** 81.

ASHERSON G.L., ZEMBALA M. & NOWOROLSKI J. (1978) The purification of specific anti-picryl suppressor factor which depresses the passive transfer of contact sensitivity: affinity chromatography on antigen and concanavalin A sepharose and specific elution with hapten and α-methyl-mannoside. *Immunology*, **35,** 1051.

ASHERSON G.L., ZEMBALA M., THOMAS W.R. & PERERA M.A.C.C. (1980) Suppressor cells and the handling of antigen. *Immunol. Rev.* **50,** 3.

BAUMGARTEN A. & GECZY A.F. (1970) Induction of delayed hypersensitivity by dinitrophenylated lymphocytes. *Immunology*, **19,** 205.

BETTS R.F. & SCHMIDT S.G. (1981) Cytolytic IgM antibody to cytomegalovirus in primary cytomegalovirus infections in humans. *J. infect. Dis.* **143,** 821.

BORSOS T., CHAPUIS R.M. & LANGONE J.L. (1981) Distinction between fixation of Cl and the activation of complement by natural IgM anti-hapten antibody: effect of cell surface hapten density. *Mol. Immunol.* **18,** 683.

CUNNINGHAM A.J. & SZENBERG A. (1968) Further improvements in the plaque technique for detecting single antibody-forming cells. *Immunology*, **14,** 599.

FULLER T.C. & WINN H.J. (1973) Immunochemical and biologic characterization of alloantibody active in immune enhancement. *Transpl. Proc.* **5,** 585.

GLIMCHER L.H., HAMANO T., ASOFSKY R., HEBER-KATZ E., HEDRICK S., SCHWARTZ R.H. & PAUL W.E. (1982) I region-restricted antigen presentation by B cell-B lymphoma hybridoma. *Nature (Lond.)*, **298,** 283.

GRANT C.K. (1976) Complement origin determines lytic activity of antibodies to nucleated target cells. Comparison of common complement sources. *Transplantation*, **21,** 323.

GREENE M.I., SUGIMOTO M. & BENACERRAF B. (1978) Mechanisms of regulation of cell-mediated immune responses. I. Effect of the route of immunization with TNP-coupled syngeneic cells on the induction and suppression of contact sensitivity to picryl chloride. *J. Immunol.* **120,** 1604.

KONTIAINEN S. & MITCHISON N.A. (1975) Blocking antigen–antibody complexes on the T-lymphocyte surface identified with defined protein antigens. I. Lymphocyte activation during *in vitro* incubation before adoptive transfer. *Immunology*, **28,** 523.

MAGUIRE H.C., FARIS C. & WEIDANZ W. (1979) Cyclophosphamide intensified the acquisition of allergic contact dermatitis in mice rendered B-cell deficient by heterologous anti-IgM antisera. *Immunology*, **37,** 367.

MITCHELL F., HUMPHREY J.H. & WILLIAMSON A.R. (1972) Inhibition of secondary anti-hapten responses with the hapten conjugated to type 3 pneumococcal polysaccharide. *Europ. J. Immunol.* **2,** 460.

PTAK W. & GERSHON R.K. (1982) Immunological agnosis: a state that derives from T suppressor cell inhibition of antigen-presenting cells. *Proc. natn. Acad. Sci. (U.S.A.)*, **79,** 2645.

RUBINSTEIN P., DECARY F. & STREUN E.W. (1974) Quantitative studies on tumour enhancement in mice. I. Enhancement of sarcoma I induced by IgM, IgG1 and IgG2. *J. exp. Med.* **140,** 591.

THOMAS D.W. & SHEVACH E.M. (1978) Nature of the antigenic complex recognized by T lymphocytes. VI. The effect of anti-TNP antibody on T cell responses to TNP-conjugated macrophages. *J. Immunol.* **121,** 1145.

THOMAS W.R., EDWARDS A.J., WATKINS M.C. & ASHERSON G.L. (1980) Distribution of immunogenic cells after painting the contact sensitizers fluorescein isothiocyanate and oxazolone. Different sensitizers form immunogenic complexes with different cell populations. *Immunology*, **39,** 21.

THOMAS W.R., WATKINS M.C., WOOD P.J. & ASHERSON G.L. (1978) Antibody responses to contact sensitizing agents: effect of sensitized cells. *Immunology*, **34,** 411.

TUCKER M.J. & BRETSCHER P.A. (1982) T cells co-operating in the induction of delayed-type hypersensitivity act via the linked recognition of antigenic determinants. *J. exp. Med.* **155,** 1037.