Immunopotentiation

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Immunopotentiation
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Introduction

SIR PETER MEDAWAR

Clinical Research Centre, Harrow

For the past twenty years the control of the immune response has been virtually equated to immunosuppression because the great goal of applied immunology has been the transplantation of tissues between individuals.

With the discovery of tumour immunity—that even autochthonous tumours may excite a cell-mediated immune reaction directed against themselves (Foley 1953; Prehn & Main 1957; Old et al. 1962; Klein et al. 1960; Habel 1961; Sjögren et al. 1961)—the focal point of immunological control has changed from immunosuppression to immunopotentiation, and correspondingly the great prize of applied immunology has become the prevention and control of malignant growth.

Nevertheless many contributors will probably emphasize that what is called for is a very exact ‘immunoregulation’ in which some arms of the immune response are strengthened and others diminished in power, as the circumstances may require. Thus the work of Turk & Poulter (1972a, b) and Turk et al. (1972) makes it increasingly clear that the potentiation of cell-mediated immunity should be accompanied by inhibition of the humoral antibody response, which is also tumour-specific.

Immunopotentiation must obviously be non-specific in the first instance because we have no a priori knowledge of what the tumour neo-antigens are and must therefore be forearmed against them all, so far as such a thing is possible.

I nevertheless emphasize with some hope of being confuted that non-specific potentiation is of a much lesser order of magnitude than specific potentiation. Thus, in the context of skin allografts, which provide perhaps the most trustworthy measure of cell-mediated immunity, no amount of non-specific potentiation can really approximate the degree of sensitization that may be achieved by specific immunization against a skin allograft. (I am comparing in my mind the behaviour of a typical ‘second-set’ skin allograft with the behaviour of an allograft after intensive non-specific potentiation of immunity.)
By the end of the symposium I hope we shall have a clearer idea of what tests should be applied to evaluate a supposedly immunopotentiating agent. Thus if someone produces a vial containing a pure white crystalline compound, or more likely a brown amorphous rather treacly compound, said to be a powerful immunopotentiating agent, we should know what procedures to adopt in order to substantiate or challenge the claim. Among these will surely be the kinetic tests of lymphocyte behaviour introduced in recent years by Lance & Taub (1969).

The history of the concepts underlying immunopotentiation is to quite a high degree embodied in the people attending this conference, including Dr Dresser with his fundamental analysis of adjuvants and adjuvanticity and Professors Mathé and Halpern with the introduction of bacterial vaccines into the clinical treatment of tumours. We look forward to learning about their latest work, and that of the other contributors to this symposium.

References

Foley, E. J. (1953) Antigenic properties of methylcholanthrene induced tumours in mice of the strain of origin. Cancer Res. 13, 835
The cellular targets for the action of adjuvants: T-adjuvants and B-adjuvants

D. W. DRESSER and JENNIFER M. PHILLIPS

The National Institute for Medical Research, London

Abstract Experiments with thymectomized mice have made it possible to find T- and B-cell orientated adjuvants. *Bordetella pertussis* organisms, which were previously known to have activity towards macrophages, were shown to be orientated towards both T and B cells, whereas lentinan acts on T cells and lipopolysaccharide from *S. typhosa* on B cells. A simple assay, using normal mice, is described for ascertaining the cellular orientation of adjuvants. The hypothesis that all adjuvants stimulate cell division, and that the heterogeneity of the adjuvant effect is due to activity against cells at different stages of differentiation, is discussed.

Substances incorporated into or injected concomitantly with an antigen which potentiate the ensuing immune response are called immunological adjuvants. A classical example of such material is the admixture of a solution or suspension of the antigen in aqueous medium with a water-in-oil emulsion containing mycobacterial lipids in the oil phase (see Freund 1953): this is Freund's complete adjuvant (FCA). Other well-known adjuvants are insoluble particles of aluminium phosphate on to which the antigen has been adsorbed (Ramon 1926; Glenny *et al.* 1926) and the admixture of antigen and bacterial endotoxin (Johnson *et al.* 1956).

Freund (1953) suggested, with particular reference to the action of FCA, that adjuvants might act in one or more of three ways. First, the potentiating stimulus may be due to a protracted antigenic stimulus resulting from the slow release of antigen from the depot in the water-in-oil emulsion (see Freund 1951). Second, the reticuloendothelial system could be stimulated by adjuvant. This could involve more rapid ingestion of the antigen (Rupp *et al*. 1960) or more effective processing of antigen leading to more extensive lymphocyte stimulation (Unanue *et al*. 1969; Allison & Davies 1971). Freund's third category of a generalized stimulation of the immune response would probably include the
direct action of the adjuvant on the lymphocytes concerned in the immune response, the effect of adjuvant at different times during the course of cellular differentiation, the anatomical site of interaction between cells and between cells and antigen and, finally, the possible importance of the formation of granulomata.

It has recently been shown that for a humoral immune response to follow the injection of many antigens, two different kinds of lymphocyte must interact. The thymus is involved in the development of one kind (T cell) which helps cells of another line, derived from the bursa of Fabricius in birds or the bone marrow in mammals (B cells), to produce humoral antibody (Claman et al. 1966; Davies et al. 1967; Taylor et al. 1967; Mitchell & Miller 1968; Miller & Mitchell 1969; Taylor 1969). In many circumstances (Rajewsky & Röttlunder 1967; Mitchison 1967) but not all (Katz et al. 1971) the T cell can be shown to have a specific relationship to at least one determinant on the antigenic molecule. It is clear therefore that any discussion of the immune response, including (a) immune paralysis (tolerance) (Chiller et al. 1970) and (b) adjuvant action (Allison & Davies 1971; Dresser 1972) must include a consideration of these effects on T and B cells and perhaps also macrophages, independently and as parts of an interacting society of immunocytes.

The requirement for interaction between T and B cells focuses interest on a physiological effect of adjuvants, which may assist T cells, B cells and macrophages to come into contact with each other in lymph nodes draining a site of infection or of adjuvant injection. Circulating lymphocytes are trapped in the paracortical area of lymph nodes draining such a site (Taub et al. 1970; Taub & Gershon 1972). A large proportion of these circulating cells are T cells. Whether or not circulating B cells play a role in the immune response in a local lymph node, it is clearly of importance that adjuvant can help to bring together, in a suitable environment, all the ingredients of such a response. The trapping mechanism may be mediated through macrophages (Dr E. M. Lance, personal communication) or through a direct effect of the adjuvant on the littoral cells of the efferent sinuses which swell to block lymph flow (Smith & Wood 1949; Florey 1969).

An antigen which is capable of eliciting an immune response without the need of additional adjuvant can be considered as possessing its own intrinsic adjuvanticity and may conveniently be called an immunogenic antigen. In contrast a non-immunogenic antigen will elicit a state of specific immunological paralysis (tolerance) unless the antigen is polymerized or a substance with adjuvant-like properties (extrinsic adjuvanticity) is injected at the same time as the antigen (Dresser 1961, 1962; Taub et al. 1970). Classical adjuvants therefore can be considered as being substances with extrinsic adjuvanticity (Golub
& Weigle 1967; Finger et al. 1968; Dresser 1968a, b) and whatever other activities a substance with such adjuvanticity may have in an animal injected with a non-immunogenic antigen, the first is to prevent the antigen from inducing immunological paralysis. It is possible that an antigen-sensitive cell (ASC) requires two stimuli or signals if it is to start dividing and differentiating into cells which produce antibody (Bretscher 1972). A cell which receives only the specific signal resulting from the binding of monomeric or monovalent antigen becomes ‘paralysed’ or is killed (Dresser 1962). The two signals necessary for the induction of immune differentiation might be provided either by an ASC binding an antigen which possesses intrinsic adjuvanticity or by such a cell binding antigen and at the same time receiving an ‘insult’ which results in the physiological response which is the essential first step towards immune differentiation. This non-specifically stimulated physiological response by an ASC might be cell division, pinocytosis or a combination of both: a circumstance which might allow antigenic determinants to penetrate into the nucleus (Dresser 1970). An antigen with its own built-in adjuvant properties (intrinsic adjuvanticity) might possess this feature through being polymeric and capable of causing mechanical strain in the membrane of a cell binding that antigen (Taylor & Iverson 1971), or alternatively through toxic properties which an endotoxin, for instance, might be expected to possess and which could provide a chemical rather than a mechanical insult to the membrane of the ASC.

The action of an adjuvant helping in the cellular decision-making process, as outlined above, may be different from its action in increasing the level of immune responsiveness. The difference may lie either at the level of the kind of ‘adjuvant-like’ insult given to a cell or in differences in the timing of the adjuvant action in relation to the timing of contact between cell and antigen. The evidence cited above, stating that all classical adjuvants which potentiate the level of humoral immunity also prevent the induction of immunological paralysis, suggests that it is unlikely that the different adjuvants at present in common use show any specificity as to the stage of differentiation of the target cells. However, there is no reason to believe that T cell help cannot act in different ways: perhaps these are by ‘polymerizing’ and presenting antigen in a locally concentrated form to ASC (Taylor & Iverson 1971), or by releasing a T cell (adjuvant-like) factor, or by acting as an aggressor cell at later stages in the maturation of antibody-forming cells (Katz et al. 1971; Kreth & Williamson 1971).

It has been suggested previously (Dresser 1970) that all adjuvant activity is mediated through the stimulation of cell division. If this is so, possible different effects of adjuvants may be due to the stimulation of division in cells which are at different stages of differentiation. For instance cell division, or a marked
increase in division rate, may be stimulated in uncommitted precursor cells or among committed cells which are already on the pathway to a full expression of their immunopotential. Fig. 1 illustrates, in a simplified form, when an adjuvant, and perhaps also T cell help, might be able to influence the maturation of B cells. A somewhat similar process may take place in the specific education or sensitization of T cells (Mitchell & Miller 1968).

![Diagram of cell division](image)

**Fig. 1.** A diagram to illustrate when an adjuvant can act on a population of differentiating B cells. (1) Adjuvant can act on precursor cells before contact with antigen; (2) T cells or B-adjuvant can help to give antigen-sensitive cells the second stimulus necessary for induction of an immune response; (3) T cells or adjuvant may be able to stimulate cell proliferation, the γ1-receptor-bearing cells being more susceptible to such stimulation than μ-bearing cells. The progressive restriction in the ability of cells to produce and manifest receptors of particular H class is indicated (see Kincade et al. 1970; Anderson 1972; Greaves 1971; Lawton et al. 1972).

**EXPERIMENTS ILLUSTRATING THE EFFECT OF ADJUVANT ON THE NUMBERS OF CELLS PRODUCING ANTIBODY OF DIFFERENT CLASSES**

Studies of the response of mice to sheep red blood cells (SRBC) have been made by measuring the numbers of cells making antibody at various times after immunization, using the plaque assay originally developed by Jerne et al. (1963). Subsequent modifications to the method enable each of the five main classes of humoral antibody-producing cells in mice to be assayed independently (Dresser & Wortis 1965; Sterzl & Riha 1965). This assay, described in detail by Dresser & Greaves (1973), has been used in the experiments summarized in this
Experiments in which *Bordetella pertussis* organisms have been used as an adjuvant in mice immunized with SRBC have shown that the adjuvant effect is far greater on the γG1 class than on the γM class (Dresser *et al.* 1970; Torrigiani 1972). Furthermore, H. H. Wortis, D. W. Dresser & H. R. Anderson (unpublished) have shown in experiments using the methodology of Claman *et al.* (1966) that the γG1 class is more T cell-dependent than the γM class, a conclusion confirmed using adult thymectomized mice reconstituted with foetal liver (T- or B mice) (Taylor & Wortis 1968; Dresser 1972). It seems that the potentiating effects of T cells and of pertussis on the five classes of mice can be ranked in order as follows: γG1 > γG2b > γG2a and γA > γM.

The effect of a concomitant intraperitoneal injection of $2 \times 10^9$ pertussis organisms on the response of CBA mice to $4 \times 10^7$ SRBC injected intraperitoneally was examined and the results are presented in Figs. 2 and 3. The time

![Graph](image)
Fig. 3. Similar to Fig. 2, but for γG2a-PFC. There are no measurable γG-PFC after the injection of pertussis without sheep red cells.

(in hours) required for the pool of antibody-producing cells to double during the initial ascending portion of each time-response curve is given; it can be seen that pertussis increases the rate of growth of the pool of plaque-forming cells (PFC), a point previously noted in the rat by Rowley et al. (1968). The results are plotted as PFC per $10^6$ spleen lymphocytes which means that overall changes in numbers of spleen lymphocytes are not apparent. However, in Fig. 4 it can be seen that there is also an increase in the number of spleen lymphocytes. At day 5 after the injection of pertussis and SRBC there may be as many as $100 \times 10^6$ more lymphocytes than at day 0, but only about $6 \times 10^6$ of these can be shown to be producing antibody. This is not incompatible with the suggestion of Perkins et al. (1969) that the pool of mostly non-dividing antibody-producing cells is fed from a pool of rapidly dividing non-producing cells. It is quite likely that this differentiation step is ‘driven’ by antigen, is related to the difference between a primary and a secondary response, and can be influenced by non-specific factors, but we shall not discuss these points here.
T AND B ADJUVANTS

Fig. 4. The effect of the intraperitoneal injection of $4 \times 10^7$ sheep red cells (S), of $2 \times 10^9$ pertussis organisms (P), and both together (P + S), on the total number of lymphocytes (Coulter Model B) in the spleens of CBA mice. Ordinate, geometric mean lymphocytes ($\times 10^{-6}$ per spleen) (Lys.).

THE ADJUVANT EFFECT OF PERTUSSIS IS MEDIATED THROUGH T AND B CELLS

Allison & Davies (1971) showed that some adjuvants can mediate their effect through T cells. The implication is that more or more active T cells give B cells a greater or more prolonged helper effect. Using thymectomized and reconstituted T- (B) mice and T+ (sham-operated control) mice with and without pertussis (P+ and P-) in a symmetrically designed experiment, we were able to show in relative terms that the adjuvant effect of pertussis was largely mediated through T cells at low doses of antigen ($4 \times 10^7$) and to a far greater extent directly on the B cells at high doses of SRBC ($4 \times 10^9$) (Dresser 1972). In Fig. 5 similar data are presented graphically. The effectiveness of the addition of pertussis, expressed as the ratio of PFC in the presence and absence of pertussis, is plotted for thymectomized (T-) and sham-operated control (T+) mice against three doses of antigen, for the $\gamma$M and $\gamma$G1 classes. The relative effect of pertussis in T- mice shows a dramatic rise with increasing dose of antigen for the $\gamma$G1 class whereas there is little change in the effect on the $\gamma$M class.

The evidence discussed so far supports the view that a high concentration of antigen plus adjuvant can by-pass a dependence on T cell help and confirms the conclusion that pertussis can act directly on B cells. In Fig. 6 a more complicated analysis is made, in which an Index of Interaction (I.I.) between T cells and
The adjuvant effect of pertussis on γM and γG1 responses to sheep red blood cells in thymectomized and reconstituted (T-) and sham-operated control (T+) mice. The adjuvant effect \( (P^+/P^-) \) is the ratio of the response (PFC/10^6 spleen lymphocytes) in mice given antigen plus pertussis to that in mice given antigen (SRBC) alone. The ordinate is the log_{10} value of these ratios and the abscissa is the log_{10} (× 4) dose of SRBC injected intraperitoneally seven days before the plaque assay.

pertussis, in potentiating the response, is plotted against the dose of antigen used. Once again there is a dramatic change for the γG1 class when there is no marked change for the γM class. The drop in the I.I. for γG1 seen with increasing dose of antigen implies that the adjuvant effects seen at high doses of antigen are considerably less dependent on the adjuvant's action on T cells, than is the case at low antigen doses.

ADJUVANTS WITH T- AND B-ORIENTATION

Chihara and co-workers have made an extensive study of an anti-tumour property of lentinan, a polysaccharide isolated from the edible mushroom *Lentinus edodes* (Chihara et al. 1969, 1970). It has been suggested that lentinan acts by potentiating cell-mediated (T cell) immunity (Maeda et al. 1971; Maeda & Chihara 1971). Dr Chihara very kindly supplied us with some lentinan which we have tested in a system similar to that outlined for pertussis in the previous section.

Andersson et al. (1972, 1973) have shown that lipopolysaccharide from *Escheri-
chia coli (LPS) is a thymus-independent antigen and furthermore has a mitogenic effect on B but not on T lymphocytes. Möller et al. (1972) have stated that LPS added to sheep red cells can convert the latter from a thymus-dependent antigen to a thymus-independent antigen; perhaps this is because the LPS acts as a B cell adjuvant.

Lentinan was dispersed in physiological saline by ultrasonication and injected intraperitoneally in a single dose of 250 μg at the same time as $4 \times 10^9$ SRBC into T+ and T- mice; in another experiment with normal mice 25 μg was injected intraperitoneally each day for five days before the injection of SRBC. Controls were injected with saline. LPS was also tested in T+ and T- mice injected concomitantly with $4 \times 10^7$ or $4 \times 10^9$ SRBC. The LPS (S. typhosa 0901) was suspended in saline using a mini-Waring blender (MSE).

The results, expressed in terms of the Index of Interaction (see Fig. 6), are presented in Table 1 for high doses of antigen and in Table 2 for low doses of antigen. A high index indicates that T cells contribute a great deal to the potentiation of the response and a low value for the index implies that antigen together with adjuvant can act directly on B cells. If it is generally accepted that high doses of antigen plus B-orientated adjuvant are required for direct activ-
TABLE 1

An Index of Interaction (I.I.) between T cells and adjuvant in the potentiation of the immune response to $4 \times 10^9$ sheep red blood cells injected intraperitoneally

<table>
<thead>
<tr>
<th>Adjuvant</th>
<th>Index of interaction</th>
<th>Spleen lymphocytes</th>
<th>$\gamma M$</th>
<th>$\gamma A$</th>
<th>$\gamma G1$</th>
<th>$\gamma G2a$</th>
<th>$\gamma G2b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lentinan 250 $\mu$g, i.p., day 0</td>
<td>0.77</td>
<td>14.7</td>
<td>$&gt; 100$</td>
<td>$&gt; 100$</td>
<td>$&gt; 100$</td>
<td>$&gt; 100$</td>
<td></td>
</tr>
<tr>
<td>Pertussis $2 \times 10^9$, i.p., day 0</td>
<td>1.41</td>
<td>0.4</td>
<td>0.05</td>
<td>0.007</td>
<td>0.3</td>
<td>0.006</td>
<td></td>
</tr>
<tr>
<td>LPS 50 $\mu$g, i.p., day 0</td>
<td>1.02</td>
<td>0.39</td>
<td>0.26</td>
<td>1.25</td>
<td>0.04</td>
<td>0.01</td>
<td></td>
</tr>
</tbody>
</table>

$I.I. = \frac{PFC \text{ Adj}^- \text{ in } T^- \text{ mice}}{PFC \text{ Adj}^+ \text{ in } T^+ \text{ mice}} = \frac{PFC}{10^6 \text{ spleen lymphocytes}}$.

(I.I. derived from Dresser 1972.)

TABLE 2

An Index of Interaction (I.I.) between T cells and adjuvant in the potentiation of the immune response to $4 \times 10^7$ sheep red blood cells injected intraperitoneally

<table>
<thead>
<tr>
<th>Adjuvant</th>
<th>Index of interaction</th>
<th>Spleen lymphocytes</th>
<th>$\gamma M$</th>
<th>$\gamma A$</th>
<th>$\gamma G1$</th>
<th>$\gamma G2a$</th>
<th>$\gamma G2b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pertussis $2 \times 10^9$, i.p., day 0</td>
<td>1.40</td>
<td>0.6</td>
<td>10.0</td>
<td>100.0</td>
<td>14.3</td>
<td>5.0</td>
<td></td>
</tr>
<tr>
<td>LPS 50 $\mu$g, i.p., day 0</td>
<td>1.25</td>
<td>0.61</td>
<td>1.61</td>
<td>0.41</td>
<td>0.24</td>
<td>0.67</td>
<td></td>
</tr>
</tbody>
</table>

The results summarized in Tables 1 and 2 are compatible with $\gamma G1$ being the most T-dependent class and the class most affected by adjuvant. The low I.I. values observed for pertussis at high doses of SRBC (Fig. 6; Table 1) could possibly be due to the T cells being swamped by an excess of antigen and not, as suggested earlier, to an increase in activation of B cells at high doses of antigen. The high I.I. values obtained with lentinan even at high antigen dose suggest that the T cells are not swamped, strengthening the view that the drop in I.I. value seen with pertussis is due to a by-passing of the requirement for T cell help, which is seen most clearly with SRBC at low doses.

The assay for T- and B-orientation outlined above, which requires the use of thymectomized mice (T-), is cumbersome. It was thought that it might be possible to assay the orientation of an adjuvant in intact (normal) mice, and to
rely on the observation that the $\gamma G1$ response is far more T cell-dependent than the $\gamma M$ response and that B cells can only effectively be stimulated in the presence of a (B-orientated) adjuvant by high concentrations of antigen. The results of such an assay are presented in Table 3. The result is sufficiently encouraging for other adjuvants to be assayed by this system.

**TABLE 3**

Can the T- or B-orientation of an adjuvant be deduced from the differential potentiation of antibody classes at different antigen doses in normal mice?

<table>
<thead>
<tr>
<th>Adjuvant</th>
<th>LPS</th>
<th>Pertussis</th>
<th>Lentinan</th>
</tr>
</thead>
<tbody>
<tr>
<td>Orientation of adjuvant</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High Ag dose $Gadj : Madj$</td>
<td>26.9</td>
<td>9.1</td>
<td>3.6</td>
</tr>
<tr>
<td>Low Ag dose $Gadj : Madj$</td>
<td>0.6</td>
<td>2.4</td>
<td>15.2</td>
</tr>
<tr>
<td>High Ag dose : Low Ag dose</td>
<td>44.8</td>
<td>3.8</td>
<td>0.24</td>
</tr>
</tbody>
</table>

Male CBA mice, 4-6 months old, were used. The plaque assay was performed seven days after the intraperitoneal injection of $4 \times 10^7$ SRBC (low dose) or $4 \times 10^9$ SRBC (high dose).

\[ Gadj = \frac{\gamma G \text{ PFC per } 10^6 \text{ spleen cells with adjuvant}}{\gamma G \text{ PFC per } 10^6 \text{ spleen cells without adjuvant}} \]

The orientation of the adjuvants is deduced from the results presented in Tables 1 and 2.

**TIMING OF ADMINISTRATION OF ADJUVANT IN RELATION TO THE INJECTION OF ANTIGEN**

Fig. 1 (p. 6) draws attention to more than one point in time when adjuvant can act on B cells. A similar situation may exist with regard to adjuvants increasing a T cell response. It has already been pointed out (p. 5) that although all adjuvant activity might be mediated through a stimulation of cell division, the consequence of this for antigen-sensitive cells is different to that for cells already committed to producing humoral antibody.

Earlier work has shown that the adjuvant effect of pertussis is of limited duration at least in so far as the prevention of paralysis is concerned: the pertussis must be injected not more than four days before or after the antigen (Dresser 1968b). The time restriction which seems to exist for the action of pertussis in potentiating an immune response by stimulating macrophages is even greater: the injection of pertussis and antigen must be within a few hours of each other (Unanue et al. 1969). If adjuvant increases the number of cells producing antibody, it might do so by stimulating non-specific as well as
specific cells. An attempt has been made to measure the relative specificity of the adjuvant effect in mice injected with pertussis at different times in relation to the injection of antigen. Because of the many different kinds of spleen cells an absolute comparison between the PFC compartment and the total lymphocyte compartment is probably of less value than a comparison between the effects on \( \gamma M \) and \( \gamma G \) production.

The effect of injecting pertussis from five days before to five days after \( 4 \times 10^7 \) SRBC is illustrated in Fig. 7. The results are plotted for \( \gamma M \) and \( \gamma G \) in

![Graph](image)

**Fig. 7.** An attempt to answer the question: how much greater or smaller is the effect of pertussis on the splenic-PFC compartment than on the splenic-lymphocyte compartment? The ordinate is the ratio of the effect of pertussis on the number of PFC per \( 10^6 \) spleen lymphocytes to the effect of pertussis on the total number of spleen lymphocytes:

- PFC per \( 10^6 \) spleen cells with pertussis
- No. spleen cells without pertussis

\[
\frac{\text{PFC per } 10^6 \text{ spleen cells with pertussis}}{\text{No. spleen cells without pertussis}} \times \frac{\text{No. spleen cells with pertussis}}{\text{PFC per } 10^6 \text{ spleen cells without pertussis}}
\]

A value of one for this ratio indicates an equal effect on the two compartments. The time in days on the abscissa relates to the time of injection of pertussis before (−) or after (+) the injection of \( 4 \times 10^7 \) sheep red cells. Values based on means derived from assays of \( \gamma M \), \( \gamma G \) and \( \gamma G_{2a} \) made 10, 12 and 16 days after antigen injection. Four mice were assayed individually for each group on each day.
terms of the ratio of the effect of the adjuvant (P+/P-) on the PFC (PFC/10^6 spleen lymphocytes) to the effect of the adjuvant on the total spleen lymphocytes, against the time at which pertussis was injected. There is a marked difference in the effects on the γM and on the γG classes. The fall in the ratio to a value of one for γG when pertussis is injected one day before the antigen may be due to antigenic competition. Similar observations were made previously when pertussis was injected before antigens such as SRBC (Dresser 1972) or bacteriophage ΦX 174 (T.W. Tao & D. W. Dresser, unpublished). A ratio of one means that the adjuvant effect observed can be totally accounted for by an increase in the number of spleen cells.

The peaks seen for the γG responses on days 0 and +1 are compatible with a suggestion that the γG1 class may be more T cell- and adjuvant-dependent because it has to pass through more cell divisions than other classes to reach a stage of differentiation where antibody can be produced. In this context it is interesting to note that the γG1 class has a steeper dose–response curve to SRBC immunization than that of the γG2a class, which in turn is steeper than that of γM (Wortis et al. 1969; Dresser 1972). If cells destined to make antibody require continued outside triggering stimuli for mitosis to occur and if future antibody producers must divide for the differentiation process to be completed, then the dependence of a γG1 response on T cells or on high concentrations of antigen or on the presence of adjuvant can be explained. Mitosis probably occurs shortly after specific cellular induction has taken place and if this is the time when cells are most susceptible to additional mitotic stimuli, then a ready explanation is available for the very marked γG peak, seen in Fig. 7, when adjuvant was injected with, or shortly after, the antigen. It is possible that administration of adjuvant before antigen may result in the activation of a larger pool of antibody precursors which would be equally beneficial to the γM and γG classes. The data in Fig. 7 for days –5 and –3 could be interpreted as support for this hypothesis.

CONCLUSION

It is possible that adjuvants act at different time points during the cellular division and differentiation pathways leading to antibody production. They might be thought of as substances which supply the necessary second stimulus which prevents a cell which is in the process of binding monomeric antigen becoming paralysed or killed (see Bretscher 1972). This effect might be mediated through stimulation of the division of antigen-sensitive cells (Dresser 1970). In addition, adjuvants potentiate the immune response, possibly also by stimulat-
ing cell division, but among cells some hours after specific commitment. It is not impossible that the two effects are the results of a non-specific ‘insult’ to the cell membrane (Munder et al. 1969; Fischer et al. 1970).

It has been shown here that at least some adjuvants can be either T- or B-cell orientated or both. Furthermore a comparatively simple assay system has been described whereby a measurement of the relative effect of adjuvant on the γM and the far more T-dependent γG classes, at both high and low concentrations of antigen, allows an assessment of the degree of T- and B-orientation of the adjuvant to be made.

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