Antigen recognition by T-cells and its suppression

Significance and origin of rosette-forming cells

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Antigen recognition is one of the most debated aspects of the immune response and its mechanism is central to most theories of antibody formation. The clonal selection theory of Burnet (1959), which seems to be widely accepted at the moment, holds that lymphocyte clones are already in existence at birth, each bearing receptors specific for one antigen. Recent studies have shown that three cell populations are involved in immune responses, at least for some antigens:

1. Macrophages;
2. Thymus-dependent (T) lymphocytes;
3. Bone-marrow-dependent (B) lymphocytes.

The existence and mechanism of co-operation of these three cell types are still matters of discussion. One of the most debated points, and one which will interest us here, is the specificity at T and B-cell levels.

Several techniques have been proposed for studying antigen binding by lymphoid cells, the most frequently used being bacterial adherence, autoradiography of cells having bound isotope-labelled antigens and, especially, immunocytoadherence (the rosette test), which was described by Nota, Liaopoulos-Briot, Stiffel, and Biozzi (1964) and Zaalberg (1964) and developed by Biozzi, Stiffel, Mouton, Bouthillier, and Decreusufond (1968). This antigen recognition in vitro has been shown to be a model of recognition in vivo. In other words, cells which bind antigens in vitro are the same as those which bind antigens in vivo, this being the first step in antigen recognition (Bach, Muller, and Dardenne, 1970). Antigen binding can be prevented in several ways, including removal of antigen, neutralization of the receptors responsible for the binding and membrane modification making the receptors unavailable for antigen.

The Clones

The clonal selection theory of Burnet (1959) is supported by the fact that antibody-forming cells (as studied by the local haemolysis in gel technique) are restricted in specificity, class, and allotype (Dubiski and Fradette, 1966). One particular plaque-forming cell generally produces antibodies of one class, one allotype, one specificity, and one affinity, with a few exceptions (Mäkelä and Cross, 1970; Liaopoulos, 1971). While this is suggestive of clonal populations of lymphocytes, direct proof of the existence of clones before immunization is needed to support the clonal selection theory. Such direct proof has been afforded by a number of workers who have been able to remove one clone of a given specificity from a population of normal lymphoid cells, leaving them unresponsive to the particular antigen. Three main methods have been applied for this purpose: cell retention on antigen-coated columns, specific killing by isotope-labelled antigens, and removal of rosette-forming cells by centrifugation on gradient.

The first experiments were those of Wigzell (1970), showing that it was possible to deplete a cell population of its antigen-sensitive lymphocytes by passing the cells down an antigen-coated column. Cells with high affinity are retained more easily than cells with low affinity. The retention is inhibited by the presence of free antigen in the column. These experiments were done primarily with immune cells but have also been performed with normal cells. This system works for both thymus-dependent antigens (e.g. serum albumin), against which the immune response needs the presence of the thymus, and for thymus-independent antigens (e.g. polyvinyl pyrrolidone). The experiments of Truffa-Bachi and Wofsy (1970), using a different type of antigen-coated column, have confirmed those of Wigzell (1970) and
have provided a method of eluting highly specific cell populations.

The experiments of Ada and Byrt (1969) and of Humphrey and Keller (1971) showed that it was possible to kill specifically antigen-sensitive cells when the particular antigen was heavily labelled with an isotope and mixed with the cells. In brief, Ada and Byrt mixed normal spleen cells with 131I-labelled flagellin A and non-labelled flagellin B for 30 min. at 40°C. These cells were used for the reconstitution of irradiated mice, which then proved to be unresponsive to flagellin A, while retaining normal responsiveness to flagellin B. Similarly, Dutton and Mishell (1967) have shown with the hot-pulse technique that there is a population of cells specifically involved in antigen recognition towards sheep erythrocytes which is completely different from that reacting towards red blood cells of other species. Lastly, affinity labelling has been used (Plotz, 1969; Segal, Globerson, Feldman, Haimovich, and Givol, 1969) to make receptors specifically unreactive.

We have performed experiments in which we have removed from normal cell populations those cells forming rosettes with sheep erythrocytes. The remaining cells were then unresponsive to this antigen. The starting hypothesis was that cells recognizing heterologous red cells formed rosettes. In order to obtain depletion of spontaneous rosette-forming cells (sRFC) from normal spleen cells, we have mixed the latter with red cells so as to form the rosettes and then put the cell suspension on a Ficoll/Triosil mixture. Such a mixture is generally used to separate lymphocytes from red cells (Bach, Muller, and Dardenne, 1970). After centrifugation, in our particular experiment, rosette-forming lymphocytes which were coated with red cells behaved like erythrocytes and went through the Ficoll, thus becoming separated from the other lymphocytes. The immunological competence of the depleted population was tested by restoring animals made immunologically anergic by a cyclophosphamide injection (300 mg. intraperitoneally). The results (Bach, Muller, and Dardenne, 1970) showed that depletion of cells forming rosettes with sheep erythrocytes induced unresponsiveness against sheep red blood cells. This was true both at the humoral level (haemagglutinins) and the cellular level (plaque-forming cells, rosette-forming cells). As controls, we showed that the passage on Ficoll in the presence of red cells but without rosette formation, and rosette formation and incubation at 37°C in Ficoll but without centrifugation, did not induce a similar unresponsiveness, thus excluding tolerance induction in vitro.

Similar results have been obtained in immunized mice. The procedure was similar to that described above for normal cells, the only difference being that immune cells were taken instead of normal cells. It is thus suggested that memory cells for heterologous erythrocytes bind antigen and form rosettes.

These experiments and the others quoted above strongly suggest that there are specific clones in the unimmunized mouse. The size of these clones is difficult to estimate. Several figures have been proposed for sheep red blood cells (SRBC). Makino-dan and Albright (1963), using cell dilution techniques, proposed 150 antigen-sensitive cells per spleen; Playfair, Papermaster, and Cole (1965) and Kennedy, Till, Siminovitch, and McCulloch (1966), by the colony technique, gave figures of 5,000; Biozzi and others (1968), by extrapolating the kinetics of rosette-forming cells (RFC), proposed similar figures of 2,000 to 6,000. Looking at spontaneous RFC, we have found significantly higher figures. Similarly, Byrt and Ada (1969) and Humphrey and Keller (1971) also obtained high figures, around 1 antigen-binding cell per 1,000 cells. These two kinds of evaluation may be reconciled if one accepts that antigen-binding cells represent a mixture of macrophages, B and T-cells (as will be detailed later). Moreover, it is known that antigen-binding cells have a wide range of affinity and that probably only high affinity cells are involved in one particular response. In fact, when rosettes are formed with a low erythrocyte/leucocyte ratio, fewer RFC are found (~0.1 per cent. for a ratio of 0.1) (Bach, Reyes, Dardenne, Fournier, and Muller, 1971). This explains why, when looking at rosettes or antigen-binding cells, one should consider only the high affinity B or T-cells as the clone involved in one response. On the whole, it seems reasonable to evaluate the size of the clones at about 1 per 10^4 lymphocytes for red cells (which have a complex mosaic of antigens) and perhaps five to twenty times less for simpler antigens.

**Cellular co-operation**

It has been shown for heterologous red cells and for some other antigens, such as serum proteins, that antibody production needs the co-operation of two populations of lymphocytes: one having been processed by the thymus (T-lymphocytes), the other coming directly from the bone marrow (B-lymphocytes). Macrophages also play a role at the first step of the immune response by transmitting 'super antigens' to lymphocytes. The mechanism of this cellular co-operation is still uncertain. From all the published work the following scheme may be presented: the T-cells recognize the antigen and present it to the B-cells. It might be, at least in certain systems, that the T-cells will recognize antigenic determinants different from those recognized by the B-cells. In a hapten-carrier system, the T-cells would recognize the carrier and the B-cells...
the hapten. T-cells would mainly have a function of antigen concentration on B-cells (Miller and Mitchell, 1969; Mitchison, 1971). The mechanism of antigen concentration by T-cells is still a matter for speculation. One possibility is that the T-cells have a lower threshold of sensitivity to antigen with a wider range of specificity. The B-cells would produce the anti-hapten antibody. The effects of neonatal thymectomy, which suppresses the T-cell population, can be overcome by the injection of high doses of antigen, short-circuiting the simple helper function of T-cells for B-cells. In the first days after antigen stimulation T-cells divide, as has been shown by Davies (1969) using a chromosome marker. Some of the T-cells can recirculate, which differentiates them from B-cells. The main methods used to deplete T-cells are neonatal thymectomy, antilymphocyte serum (ALS) treatment, and thoracic duct drainage. B-cells are responsible for antibody production, as shown by chromosome or antigenic markers (Miller and Mitchell, 1969; Nossal, Cunningham, Mitchell, and Miller, 1968). The mechanism of antigen recognition is not well understood. One of the main problems is the location of specificity. Is specificity the same at the B and T level or is it peculiar to one of these two populations?

**SPECIFICITY AT THE B-CELL LEVEL**

There are several arguments in favour of this. DNA synthesis can be specifically induced in bone marrow cells and can be specifically eliminated by passage of the cells on antigen-coated columns (Singhal and Richter, 1968; Wigzell, 1970). Bone marrow RFC are specific for the antigen (Bach, Reyes, and others, 1971) and the depletion of RFC from the bone marrow makes it incapable of co-operating with the thymus (Brody, 1970). Moreover, in a carrier-hapten system, Wigzell (1970) has shown that the depletion involves anti-hapten cells rather than anti-carrier T-cells. Also, responses against antigen not needing the presence of thymus can be suppressed by passage of the cells on antigen-coated columns (Wigzell, 1970). Lastly, Playfair (1969), Chiller, Habicht, and Weigle (1970), and Argyris (1968) have shown that, when a mouse is tolerant towards certain antigens, the tolerant cells can be found in the bone marrow. On the other hand, it is not possible to induce tolerance in a thymus-marrow co-operation system by incubating bone-marrow with antigens heavily labelled with radioisotope (Basten, Miller, Warner, and Pye, 1971).

**SPECIFICITY AT THE T-CELL LEVEL**

This has also been demonstrated by a number of experiments. Double transfer experiments (Claman and Chaperon, 1969; Miller and Mitchell, 1969) and the study of T-cell mitoses (Davies, 1969) have shown that stimulation of T-cells is specific. In the carrier-hapten system already mentioned, the carrier effect, which is mainly thymus-dependent, is specific for the antigen. When tolerance is induced in vivo, the thymus is made specifically tolerant and unable to co-operate with bone marrow for this antigen (Isaković, Smith, and Waksman, 1965; Miller and Mitchell, 1969; Taylor, 1969; Chiller and others, 1970). Tolerance in T-cells is easier to induce than tolerance in B-cells. Greaves (1970) has shown that, when irradiated mice are reconstituted with syngeneic bone marrow cells and semi-syngeneic thymus cells, a large portion of RFC is of thymic origin (indicated by specific inhibition using anti-H2 serum). These data indicate that specificity exists in T-immune cells, although this specificity might be less evident than in B-cells. One of the main arguments in favour of this low specificity is the high number of antigen-sensitive cells found in a pure T-cell system, like graft versus host (GVH) (Nisbet and Simonsen, 1967) and mixed lymphocyte culture (MLC) (Wilson, Klyth, and Nowell, 1968). This high percentage, 1 to 2 per cent. of lymphocytes, is a matter of speculation. It is fundamental to the new theory proposed by Jerne (1971) for antibody formation.

**Origin of rosette-forming cells (RFC)**

Rosette-formation represents an interesting model for the study of the origin of antigen-sensitive cells; as rosette-forming lymphocytes present in bone marrow, spleen, or thymus are specific for an antigen (Bach, Reyes, and others, 1971), determination of the origin of RFC gives information about the specificity of the lymphocyte population.

**Bone marrow sRFC**

These bear immunoglobulin receptors for the antigen. They probably include:

1. Cells co-operating with thymus, since their removal specifically induces inability of bone marrow cells to co-operate with thymus (Brody, 1970);
2. Precursors of T-cells, since incubation of bone marrow cells with thymosin produces cells able to form rosettes and having the attributes of T-cells, namely theta antigen and sensitivity to azathioprine and ALS (Bach, Dardenne, Goldstein, Guha, and White, 1971).

**Thymus sRFC**

These are present only in small numbers in normal thymus (20 to 100 per 10⁶ cells). However, after an injection of hydrocortisone, whereas the total number of thymus cells is drastically reduced, the percentage of RFC increases equivalent to that of the spleen (Bach and Dardenne, 1971a). This is a
general feature of thymus cells, since all the thymus cells able to induce graft versus host reaction (Blomgren and Andersson, 1969; Cohen, Fischbach, and Claman, 1970) and to co-operate with bone marrow (Andersson and Blomgren, 1970) are also present in this hydrocortisone-resistant pool.

**Spleen sRFC**

There are also T-RFC in the spleen, since the unresponsiveness induced by removal of sRFC can be partially reconstituted by thymus cells (Bach and Dardenne, 1971a). Such spleen sRFC are present at a normal level in neonatally thymectomized animals (Bach and Dardenne, 1971a) but are then insensitive to azathioprine and ALS (Bach and Dardenne, 1971b), suggesting that T-cells possess receptors before being processed by the thymus. Moreover, 75 per cent. of spleen sRFC bear the theta antigen (Bach, Muller, and Dardenne, 1970; Bach and Dardenne, 1971a) which is characteristic of T-cells, as described below.

Adult thymectomy eliminates theta-positive azathioprine sensitive RFC within 5 days, suggesting that spleen T-RFC are short lived (Bach, Dardenne, and Davies, 1971).

**Theta-negative spleen sRFC**

These are probably antigen-sensitive B-cells, since the unresponsiveness induced by removal of anti-sheep sRFC is partially reconstituted by bone marrow cells. This reconstitution with bone marrow cells becomes total when studying anti-chicken RFC (Bach and Dardenne, 1971a).

**Lymph node sRFC**

These are 90 per cent. theta-positive. They are probably long lived and they recirculate since they are not modified by adult thymectomy. They disappear 6 hours after an injection of ALS. (Our unpublished data.)

**T-cell markers**

Theta (θ) is an iso-antigen, genetically determined in mice according to two alleles, theta AKR and theta C3H, Schlesinger and Yron (1970) and Raff (1969) have shown that theta is a marker of T-cells. It is present in thymus and brain and also in thymus-derived cells. Neonatal thymectomy and anti-lymphocyte serum (ALS) treatment lead to the disappearance of theta-positive cells in lymph nodes (Raff and Wortis, 1970; Schlesinger and Yron, 1969).

We have shown that azathioprine and ALS inhibit sRFC exactly as anti-theta serum does in all systems tested: neonatal thymectomy (Bach and Dardenne, 1971b), adult thymectomy (Bach, Dardenne, and Davies, 1971), and distribution in the lymphoid organs (Bach and Dardenne, 1971b). The superiority of azathioprine over theta as a marker of T-cells is the probable lack of species specificity of azathioprine and its possible application in man. Moreover, the use of a chemical substance avoids the difficulty of standardization needed for biological products, which is particularly true for anti-theta serum containing several categories of antibodies.

**Suppression of antigen recognition**

Antigen recognition occurs by means of antibody-like specific receptors located in the lymphocyte membrane; hence a number of experimental conditions can suppress antigen recognition: removal of antigen (injection of passive antibodies), neutralization of receptors (anti-Ig serum), and suppression of receptor availability without direct action on the receptors themselves (azathioprine, ALS). Other possibilities could be put forward, but only those listed above will be discussed here.

(1) **Removal of antigen** (Uhr and Möller, 1968; Wigzell, 1969) (Suppression of antigen recognition by passively administered antibody).

Primary antibody formation can be prevented by mixing the antigen with excess antibody before injection, or injecting antibody before, simultaneously, or even after the antigen. The phenomenon seems to be a very general one. It might represent a feed-back function of antibody on its own formation. The mechanism by which antibody synthesis is suppressed by passively administered antibody involves primarily a blocking of antigen site and elimination of the antigen in the form of an antigen-antibody complex. Some authors have suggested a central action at the lymphoid cell level. In favour of this hypothesis is the observation that incubation of normal spleen cells with anti-red blood cell antibodies makes these cells unresponsive to red cells. However, these results have been contradicted in other experiments. The action of passively administered antibody is specific for the antigen and resides in the Fab fragment of the immunoglobulin (Ig). Inhibition of antigen recognition can be obtained even when injecting the antibody several days after the antigen, indicating that the majority of antibody-producing cells during the early stage of the immune response are short-lived in the absence of antigen. Another point which merits comment is the much greater efficiency of high affinity antibodies in suppressing the immune response, which fits with the antigen removal hypothesis generally accepted to explain this suppression (Uhr and Möller, 1968).

7S antibodies (IgG) which are usually more avid than 19S antibodies (IgM) are the more efficient in suppressing immune responses. The main clinical application of antibody-induced immunosuppression is the injection of anti-D antibodies to pregnant women, but enhancement in kidney grafts is also being attempted.
(2) Antigen recognition by T-cells and its suppression

The receptors responsible for antigen recognition, present on the surface of antigen-sensitive cells, have been extensively studied in several situations. When anti-allotype antibodies are injected into pregnant mice, the newborn are not able to produce antibodies of the allotype that they have in their genome and against which the anti-allotype serum is directed (Dubiski and Fradette, 1966). Primary stimulation in vitro against SRBC (Greaves, 1970) or against allogeneic lymphocytes in the mixed lymphocyte reaction (Greaves, Torrigiani, and Roitt, 1969) can be inhibited by anti-light chain antibodies; moreover, in Wigzell's system of antigen-coated columns, the retention of specific cells is abolished by preincubation of the cells with anti-Ig antibodies (Greaves, 1970). Lastly, the graft versus host reaction can be transferred to irradiated mice but the transfer may be prevented if the cells are incubated with light chain antibodies before transfer (Mason and Warner, 1970). The nature of the Ig responsible for this antigen recognition system is not known. It seems that, for delayed hypersensitivity or transplantation immunity, only anti-light chain antibodies are efficient in inhibiting the response, whereas in humoral responses, anti-heavy chain sera are also efficient. In fact, the two antigen-binding reactions described above (isotope-labelled antigen binding and rosette formation) are at present the best tools for the study of receptors of antigen recognition. Greaves (1970) has shown that, in normal animals, RFC are inhibited only by anti-light chain sera, no inhibition being obtained with antisera directed against heavy chains. This observation is in contrast with what is observed with immune RFC, where a large proportion of the cells is inhibited by anti-μ chain sera (at the early stage of the response) and anti-gamma chain sera (at the late stages of the response). Warner, Byrt, and Ada (1970) have obtained some slightly different results with inhibition of flagellin-binding cells, where anti-μ sera proved to be efficient in normal mice. On the other hand, Basten, and others (1971) have obtained inhibition of bovine serum albumin-binding cells in the thymus with anti-light chain sera only.

On the whole it is possible to inhibit immune reactions by incubating normal or immune sensitive cells with anti-Ig serum. For B-cells it appears that inhibition can be obtained by both anti-light chain and anti-heavy chain sera (μ chain especially). For thymus derived (T) cells inhibition is obtained only with anti-light chain sera. No positive results have been reported for anti-heavy chains. One possibility is that receptors on T-cells have heavy chains of a new class, IgX; another is that the antigenic portion of the Ig receptor is buried in the lymphocyte membrane. Some authors doubt the presence of antibody-like receptors on T-cells. Our finding that hydrocortisone-resistant thymus sRFC, which are probably pure specific T-cells, are inhibited by anti-Ig antisera (Bach and Dardenne, 1971a) is in favour of the existence of T-cell receptors.

(3) Antilymphocyte serum (ALS)

ALS inhibits cell-mediated immune responses and those humoral antibody responses depending on the presence of the thymus (Bach, 1970a). The inhibition of such humoral antibody production is obtained only when ALS is injected before the antigen or simultaneously. ALS appears to act mainly on T-cells (at least at low dosage), since:

(i) Immuno-incompetence induced by ALS treatment can be partially reconstituted by injection of thymus cells (Martin and Miller, 1968) or thymosin, a cell-free thymus extract (Quint, Hardy, and Monaco, 1969);

(ii) Transfer experiments show that antigen-sensitive cells are more readily inactivated than antibody-producing cells (Möller and Zukoski, 1968);

(iii) ALS immunosuppressive action is enhanced by adult thymectomy (Monaco, Wood, and Russell, 1966);

(iv) ALS treatment induces a selective depletion of thymus-dependent areas in lymph nodes and spleen (Taub and Lance, 1968);

(v) Lastly, ALS is mostly active on cell-mediated immune responses, much more than in humoral antibody responses, which are mainly dependent on B-cells (Lance, 1970).

The mechanism of inactivation of T-cells by ALS is a matter of debate. It has been suggested (Lance, 1970) that ALS could influence T-cells much more readily than B-cells because T-cells are more accessible in the peripheral blood and are renewed more slowly than B-cells. This is supported by the fact that ALS is found in high concentrations in the peripheral blood, whereas it is at a lower concentration in the central lymphoid organs. Elimination of T-cells might occur by cytotoxicity or, alternatively, by opsonization as has been demonstrated in vitro and in vivo.

Alternative explanations include antigen competition, sterile activation and, especially, 'blind-folding': ALS could coat the lymphocytes and thus prevent them from recognizing the antigen. Data on rosette formation support this last hypothesis.

All ALS studied inhibit rosette formation (Bach, 1970c; Bach, Dardenne, Dormont, and Antoine, 1969; Bach and Antoine, 1968). Some inhibition is obtained without complement, but this is enhanced by the presence of complement. The percentage of

antilymphocyte serum. Some authors doubt the presence of antibody-like receptors on T-cells. Our finding that hydrocortisone-resistant thymus sRFC, which are probably pure specific T-cells, are inhibited by anti-Ig antisera (Bach and Dardenne, 1971a) is in favour of the existence of T-cell receptors.

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ALS-sensitive spleen cells is about 75 per cent.; inhibition appears after 60 min. The complement is only needed for its first two components, in contrast with cytotoxicity which needs all nine components of complement (Bach, Gigli, Dardenne, and Dormont, 1972). RFC from normal mice are much more sensitive to ALS than those from immunized mice. T-RFC from thymus and spleen are more sensitive than spleen B-rosettes and bone marrow rosettes (Bach and Dardenne, 1971b).

In summary, ALS could act by eliminating some of the T-antigen sensitive lymphocytes by opsonization (C1, C4, C2, C3), by cytotoxicity, and perhaps by inactivating the others by blind-folding, i.e. coating as in rosette inhibition with C1, C4. Such a scheme would be compatible with the fact that ALS retains all its action in C5-depleted mice (Barth and Carroll, 1970; Cinader, Jeejeebhoy, Koh, and Rabbat, 1971).

(4) AZATHIOPRINE
This drug inhibits rosette formation after 90 min. incubation at 37°C. (Bach and Dardenne, 1971c; Bach, Dardenne, and Fournier, 1969). This inhibition is total for high concentrations, but only 75 per cent. RFC are inhibited at lower concentrations. Azathioprine-sensitive cells have proved to be T-cells, since RFC lose their sensitivity to azathioprine after neonatal thymectomy (Bach and Dardenne, 1971b). Rosette inhibition by azathioprine is reversible within 20 to 30 min. which is not compatible with inhibition of receptor synthesis, but rather with modifications of the lymphocyte membrane, making the receptor no longer available for the antigen (Bach and Dardenne, 1971c). As with ALS, RFC from unimmunized animals are more sensitive to azathioprine than RFC from immunized animals. Bone marrow RFC are less sensitive to azathioprine than spleen or thymus RFC but can acquire identical sensitivity after incubation with purified thymosin, a thymic hormone (Bach, Dardenne, Goldstein, and others, 1971). As RFC found in normal non-immunized animals are antigen-recognizing cells (Bach, Muller, and Dardenne, 1970), and as azathioprine-sensitive cells are theta-positive T-cells (Bach and Dardenne, 1971b), one may suggest that azathioprine is immunosuppressive in vivo by virtue of its action on antigensensitive T-cells. This hypothesis is corroborated by the fact that azathioprine suppresses thymidine incorporation in mixed lymphocyte culture, which is a pure T-cell reaction, and that this inhibition is obtained only when azathioprine is put in the culture before the 24th hour (Bach and Bach, 1972). This action of azathioprine on antigen recognition by T-cells is in contrast with the antiproliferative action suggested by Berenbaum (1967). However, the main argument used by Berenbaum (the optimum antimetabolite action when injected 1 to 2 days after antigen stimulation) is not valid if one considers that antimetabolite action is reversible, as shown in vivo and in vitro in rosette inhibition. If given before the antigen, or on day 0 the antimetabolite would no longer be active when the antigen is still at the immunogenic level, whereas, given at day 2, the antimetabolite can suppress antigen-lymphocyte contact and prevent the response. We have shown (Bach and Fournier, unpublished) that azathioprine selectively alters the homing of T-cells: when lymph nodes are labelled with 51Cr and injected intravenously after incubation with azathioprine into normal mice, the cell portion which normally migrates to lymph nodes goes to spleen and liver, as do cells taken from neonatally thymectomized mice (Lance and Taub, 1969). The selective action of azathioprine on T-cells would explain why this drug is mostly active on cell-mediated reactions and does not prevent antibody production. Such absence of action on humoral responses might be favourable in organ transplantation, where blocking antibodies may have a very favourable action.

Clinical applications
Antigen-binding techniques can be used in man. Rosette formation appears to be much simpler (Bach, 1970b) than autoradiography after antigen labelling. The main applications of the rosette technique have been in rheumatoid arthritis (Bach and Delbarre, 1968; Bach, Delrieu, and Delbarre, 1970), thyroiditis (Perrudet-Badoux and Frei, 1969), drug allergy (Cruchaud and Frei, 1967), glomerulonephritis (Mahieu, Dardenne, and Bach, 1971), pigeon breeder's disease (Bach, Fournier, Texier, Dardenne, Laborde, and Drouet, 1971), and anti-D immunization (Elson and Bradley, 1970). The significance of rosettes found in these conditions is not yet established. The fact that the lymphocytes come from the peripheral blood suggests that RFC might be antigen-sensitive cells rather than antibody-producing cells which do not circulate much.

Another clinical application has been evaluation of immunosuppressive potency of ALS (Bach, Dormont, Dardenne, and Balmer, 1969; Bach, 1970b; Bach and Dormont, 1971) and of immunosuppressive drugs (Bach, Dardenne, and Fournier, 1969). The rosette inhibition technique proved to be sensitive enough to detect serum metabolites of antimetabolites in normal subjects (Bach, Dardenne, and Fournier, 1969) and in pathological conditions, such as renal failure (Bach and Dardenne, 1971d) and liver diseases (Bach and Dardenne, 1971e). Moreover, we have shown that abnormal sensitivity to azathioprine may be due to slow metabolic degradation (Bach, 1971).
Antigen recognition by T-cells and its suppression

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Antigen recognition by T-cells and its suppression

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