Dr Jonathan Brostoff
(Department of Immunology, Middlesex Hospital Medical School, London W1P 9PG)

Critique of Present *in vitro* Methods for the Detection of Cell-mediated Immunity

Leukocyte Migration Inhibition

It has been widely accepted that the leukocyte migration test (LMT) in man or animals is an *in vitro* correlate of delayed hypersensitivity. The test itself involves the migration of peripheral blood buffy coat cells out of capillaries into tissue culture medium containing or lacking antigen. Positive tests are indicated by the presence of significant inhibition in the antigen containing cultures compared with the controls which lack antigen (Fig 1).

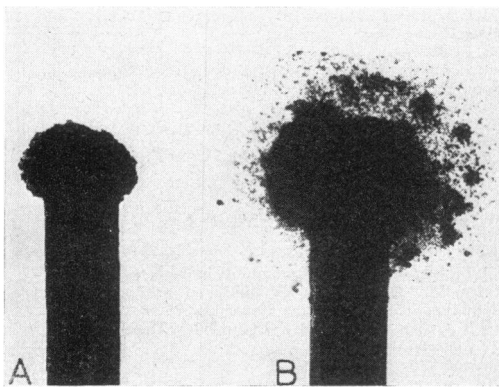

Fig 1 *Migration 'fans' in the leukocyte migration test.* A, *inhibition of migration in the presence of antigen compared with control area;* B, *in the absence of antigen*

There are some situations, however, where it is quite clear that antibody plays the major role in allowing the inhibition of the leukocytes or macrophages to occur. In other situations the presence of polymorphs is necessary for the inhibition, lymphocytes alone showing no inhibition. Without dissection of the system, the test cannot be uncritically accepted as correlating with cell-mediated immunity alone. Three situations will be discussed: (1) Animal models where antibody mediates the migration inhibition of either leukocytes or macrophages. (2) Human experiments suggesting that the LMT does not necessarily reflect cell-mediated immunity alone. (3) Where nonlymphoid cells or their supernatants seem to be necessary in man for migration inhibition to occur.

Animal Models: Leukocyte or Macrophage Migration Inhibition Mediated by Antibody

Leukocytes: Guinea-pigs immunized with thyroglobulin in complete Freund's adjuvant (CFA) develop high hæmagglutinating antibody titres against thyroglobulin. The hæmagglutinating activity is to be found in the $\gamma 1$ fraction of the serum. When normal buffy coat leukocytes are passively sensitized with the $\gamma 1$ or $\gamma 2$ fractions of serum and then allowed to migrate in tissue culture in the presence of thyroglobulin as antigen, the cells sensitized with the $\gamma 2$ antibody show migration inhibition whereas the others do not (Packalen & Wasserman 1971). In these circumstances, cell-mediated immunity plays no part in producing the migration inhibition (Fig 2) which is mediated by antibody.

Macrophages: Inhibition of migration of normal guinea-pig peritoneal exudate cells can be achieved by their passive sensitization with antibody from an immunized animal (Amos *et al.* 1967). In a more direct demonstration that cytophilic antibody itself can mediate this reaction, antibody can be eluted from sensitized guinea-pig macrophages which in turn can sensitize a normal cell population to show inhibition (Heise *et al.* 1968). These observations point to the fact that cyto-

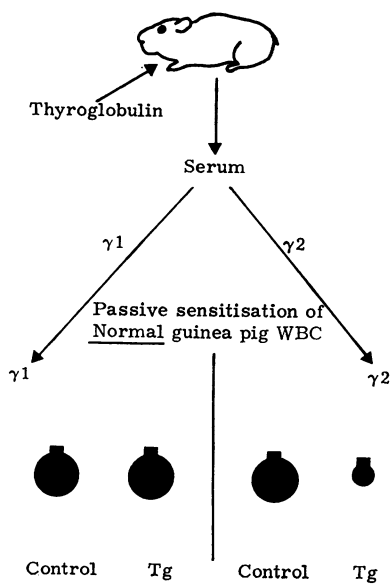

Fig 2 *Fractions of serum from a guinea-pig immunized with thyroglobulin in CFA are incubated with normal peripheral blood leukocytes. With the γ_2 fraction, these cells can be inhibited in the LMT using thyroglobulin as antigen. Tg = thyroglobulin. (Data of Packalen & Wasserman 1971)*

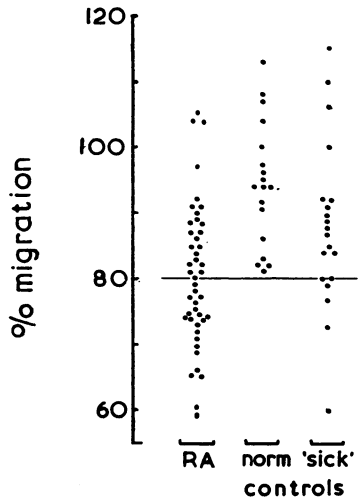

Fig 3 *Leukocyte migration with aggregated IgG. Approximately 50% of patients with rheumatoid arthritis (RA) show migration inhibition in the presence of aggregated IgG compared with healthy controls* (norm) *or patients in hospital with other diseases* ('sick')

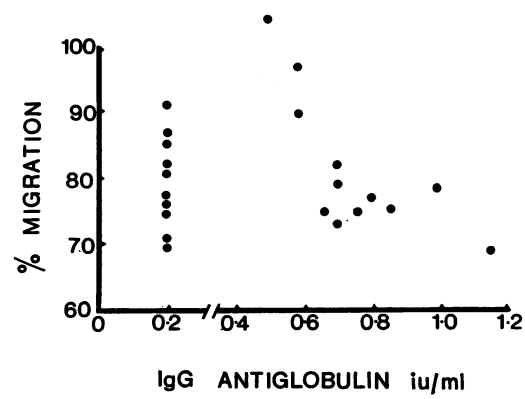

Fig 4 *Relationship between migration inhibition with aggregated IgG and serum IgG antiglobulin level. Where IgG antiglobulins are present there is a good correlation between the extent of the migration inhibition and the antibody titre* ($r=0.73$: $P<0.005$). *In the other group no such relationship is seen*

philic antibody can mediate migration inhibition but do not exclude the possibility that both antibody and delayed hypersensitivity mechanisms may be operating concurrently in the immunized animal.

Human Leukocyte Migration Inhibition

In rheumatoid arthritis (RA) the pathognomonic finding in the serum is antibody directed against host immunoglobulin, i.e. rheumatoid factor. It has been possible to show that leukocytes from patients with RA also show migration inhibition to aggregated IgG *in vitro* (Brostoff *et al.* 1973) (*see* Fig 3). The extent of this cellular reactivity is not related to the severity of the disease nor the titre of classic rheumatoid factor (IgM antiglobulin) as measured by the latex agglutination or sheep cell agglutination test (SCAT).

However, IgG antiglobulins (Torrigiani & Roitt 1967) are not detected by classical tests for rheumatoid factor and are likely to be more cytophilic than IgM antibodies. Of a group of 22 patients with rheumatoid arthritis, IgG antiglobulins were detected in 12; in these patients a good correlation was obtained between the extent of the leukocyte migration inhibition and the antibody titre (Fig 4). Therefore, crosslinking of leukocytes through immune complex formation between aggregated IgG and cytophilic antiglobulins seems a possible mechanism whereby inhibition could be obtained in these patients. In the other group where there seems to be no relationship to antiglobulin production, the LMT may well reflect an uncomplicated T cell hypersensitivity. Thus, in a relatively homogeneous group of patients using one antigen, two mechanisms may be operating in causing the migration inhibition.

Cells Involved in the LMT

Although the central cell involved in cell-mediated immunity reactions is the T lymphocyte, it has been shown that lymphocytes alone will not inhibit in the LMT. Using Brucellin in immunized human subjects, Clausen (1971) was able to show that neither lymphocytes nor monocytes alone were inhibited in the presence of antigen but when polymorphs were added to either cell population, inhibition was seen. Søborg (1970) in an elegant series of experiments was also able to show that polymorphs were necessary for migration inhibition, but that it did not matter whether the polymorphs came from an immunized or normal subject. In some instances, polymorphs alone can be inhibited from migrating without an accompanying lymphocyte population (Clausen 1971).

However, it may not be the polymorphs themselves that are important but the soluble factors released from them. Supernatants from unstimulated human or guinea-pig leukocytes in culture contain a factor that is able to inhibit the migration of macrophages. This factor is released from polymorphs and not lymphocytes and has a

molecular weight of 4000 (Stastny & Ziff 1970). This finding may not be surprising when it is remembered that an early cell to arrive at a delayed skin test site is the polymorph. Further, in neutropenic animals the appearance of mononuclear cells during the early phase of the inflammatory response is much delayed. If normal polymorphs are injected into the skin, the infiltrate returns to normal (Page & Good 1958). This is further evidence for the participation of polymorphs in delayed hypersensitivity reactions.

Conclusion

There is no doubt that the LMT is a most useful *in vitro* tool in assessing immunological reactivity in groups of patients. The exact mechanism of inhibition of migration must be worked out separately in each system of study and with each antigen before the test can definitely be said to be a correlate of cell-mediated immunity. Much valuable information has been and will be obtained through its use and careful application.

REFERENCES

Amos H E, Gurner B W, Olds R J & Coombs R R A (1967) *International Archives of Allergy and Applied Immunology* **32**, 496

Brostoff J, Howell A H & Roitt I M (1973) *Clinical and Experimental Immunology* **15**, 1

Clausen J E (1971) *Acta medica Scandinavica* **188**, 59

Heise E R, Han S & Weiser R S (1968) *Journal of Immunology* **101**, 1004

Packalen T & Wasserman J (1971) *International Archives of Allergy and Applied Immunology* **41**, 790

Page A R & Good R A (1958) *American Journal of Pathology* **34**, 645

Søborg M (1970) *Proceedings of the Royal Society of Medicine* **63**, 903

Stastny P & Ziff M (1970) *Journal of the Reticuloendothelial Society* **7**, 140

Torrigiani G & Roitt I M (1967) *Annals of the Rheumatic Diseases* **26**, 334