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CELL MIGRATION INHIBITION: AN APPRAISAL

By

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1) *The mediator concept in delayed hypersensitivity*

The importance of leucocyte emigration in the inflammatory reaction has been appreciated for more than a century (15) and the proposition that leucocyte accumulation in the inflammatory response is determined by the release of a soluble mediator is almost as longstanding; Leber (48) in 1888 having given the name "phlogosin" to a bacterial product, chemotactic for leucocytes, and Massart & Bordet (52) in 1891 having suggested that leucocytes are a source of endogenous chemotactic agents. Yet, the suggestion that local accumulation of mononuclear leucocytes in lesions of delayed hypersensitivity may be due to intervention of a soluble chemical mediator is a recent concept (9, 18). This is accounted for by the difficulties that had been encountered in the differentiation and dissociation of antigen-specific phenomena both from non-specific effects of the crude antigen preparations (e.g. toxic actions of old tuberculin *in vivo* and *in vitro*) and also, from non-specific events secondary to the antigen-specific processes (e.g. tissue necrosis in delayed hypersensitivity reactions). These difficulties have been accentuated by the absence, until recent times, of any satisfactory *in vitro* models that could be regarded as analogues of *in vivo* delayed hypersensitivity reactions.

There have been suggestions that, by analogy with the anaphylactic release of histamine in the Type I reaction following contact of antigen with the sensitized basophil, there

could be release of a corresponding *preformed* mediator following interaction of antigen with the sensitized lymphocyte (51); in each case the mediator exerting its action primarily on the microvasculature. Experimental evidence of the existence of such material was provided by Willoughby, Boughton & Schild (76) with their characterization of lymph node permeability factor (LNPF), which they distinguished from other endogenous agents capable of causing increased vascular permeability by the use of parallel bioassay.

An alternative mechanism, namely the synthesis and release of a mediator *subsequent* to antigen challenge, is a concept that has stemmed from the *in vitro* studies of cell migration inhibition. In 1932, Rich & Lewis (67) had clearly demonstrated that the presence of antigen induced an inhibition of cell migration from sensitized tissue explants *in vitro*. By use of cells packed in capillary tubes *in vitro*, George & Vaughan (38) provided an alternative to tissue explants that was more readily quantitated, and, of equal importance, more versatile. George & Vaughan's (38) conclusion that this technique provided a satisfactory *in vitro* test of cellular immunity in the guinea pig was confirmed and extended to include other antigens by David *et al.* (20) and was further strengthened by demonstration that the hapten/carrier specificity of cell migration inhibition paralleled that of skin reactivity in delayed hypersensitivity (23, 24). The versatility of the technique was soon evident; for by mixing sensitized and non-sensitized cells, it was shown that only a few cells (in some instances 2.5 per cent) in a mixed population need be specifically sensitive to influence the behaviour of the whole population (22). Further, separation of macrophages from lymphocytes permitted the demonstration that antigen could only influence the migration of macrophages in the presence of a proportion of specifically sensitized lymphocytes (9); leading in turn to the demonstration that the antigen induced inhibition of cell migration could be accounted for by an agent appearing in the culture fluid following exposure of sensitized cells to specific antigen (9, 18). Whilst these results suggested that

specifically sensitized cells derived from animals exhibiting delayed hypersensitivity interact with antigen to yield this activity, some qualification or elaboration of this statement is necessary since it is now known that these products of lymphocyte activation (28), like plant lectins (60), are themselves capable of stimulating DNA synthesis in lymphoid cells and the formation of seemingly similar biologically active material when acting on unsensitized lymphocytes. There is thus a strong possibility that an autocatalytic or positive feedback effect contributes to the production of biological activity by lymphocytes and therefore that non-sensitive as well as specifically sensitized cells can be considered likely to be involved. These lymphocyte products have in addition been shown to possess a wide range of other biological activities including the ability to cause: lymphocyte transformation; local inflammatory reactions following intradermal injection; chemotaxis; cytopathic and cytotoxic effects on target cells (see reviews (21, 62)); and the view is now widely held that such materials are mediators of cellular immunity. A comparison of the evidence supporting their status as mediators of cellular immunity with that of endocrine or neurotransmitter substances clearly illustrates the inadequate evidence upon which this presumption is currently based (53). Nevertheless, it is clear that these materials are candidates for the "title role" of mediators of cellular immunity and as such can be regarded as autopharmacological agents (17) whose immunological specificity is provided at the level of the proposed antigen receptor on the lymphocyte surface.

2) *Biological and clinical significance of macrophage migration inhibition*

Whilst skin testing remains the definitive method of establishing and measuring delayed hypersensitivity (Type IV) reactions, there are many situations in which *in vivo* testing is undesirable or prohibited. *In vitro* indicators of such reactivity are thus highly desirable, and the experiments of David, Law-

rence & Thomas (23) and David & Schlossman (24) support the contention that macrophage migration inhibition falls into this category. Measurement of macrophage or peripheral blood leucocyte migration inhibition (71) is therefore used to determine the strength or intensity of cellular immunity in situations where experimental or ethical considerations prohibit *in vivo* testing, or where it is judged to provide a more convenient system for measurement of cellular immunity.

In addition to being used with antigens employed in skin tests, it allows repeated testing of human or animal reactivity without necessitating repeated and possibly allergenic exposure to the antigen; it allows use of antigens of potential hazard e.g. pathogenic organisms (35), tumour antigens (47) and transplantation antigens (1, 34) as well as drugs in conditions of drug hypersensitivity (68).

Since the volume of blood utilized is not excessive, this type of test has the value in man of being without any associated morbidity and has undoubtedly provided useful and interesting information in a range of situations (see reviews (19, 29, 62)). However, at present, the measurement of cell migration inhibition is neither sufficiently precise nor well documented to permit its use as a sole method of clinical or experimental evaluation of Type IV hypersensitivity.

3) *What's in a name?*

These biological activities, and in particular antigen-induced inhibition of macrophage migration, having been demonstrated, it was natural that it should be a particular concern of immunologists to differentiate these activities from effects attributable to classical antibody, for it is known: that antigen-antibody complexes will similarly affect the migration of macrophages (43, 61); that in some systems addition of supplementary antigen to the culture fluid enhances or increases their biological activity (7, 73); or that biological activity only becomes evident following interaction of the antigen with molecules exhibiting immunological specificity but not identi-

fiable as immunoglobulins (2). However, such antigen-dependent materials seem restricted to particular antigen systems (e.g. PPD and β -lactoglobulin) and they do not make any contribution to the activity in other hapten-carrier systems (77). This distinction from antibody is of special relevance to the identification of mediators of Type IV reactions because of the established failure of serum antibody, as opposed to living lymphoid cells, to successfully transfer delayed reactivity into recipient animals, with the implication that antibody plays no obligatory role in the pathogenesis of delayed hypersensitivity. The natural consequence of the discovery of such biological activity (i.e. macrophage migration inhibition) and its distinction from effects produced by substances complexed, or associated with, antigen was that a term "migration inhibitory factor" was introduced by Bloom & Bennett (9) to refer to this biological activity. Whilst provision of a name for a biological activity is at some point an understandable product of necessity, the choice of this particular name has had some undesirable yet predictable consequences.

Use of a name that is both descriptive of a biological property and that, additionally, corresponds closely to the *in vivo* physiological process under investigation lends itself over readily to the assumption amongst the unwary that a cause-effect relationship *in vivo* is already an established fact. Were it to be highly probable that such a cause-effect relationship be easily established, this would be a reasonable and acceptable basis for nomenclature. Unfortunately, technical limitations are often of such magnitude that the physiological role of biologically active materials only becomes satisfactorily established years or decades after purification and synthesis of the biologically active material or materials. Hence, there is a strong possibility of irrevocably establishing in the literature a name that alludes to a biological property of no relevance to the physiological role of that substance. This is amply illustrated by endogenous biologically active peptides where for example angiotonin and hypertensin, after a protracted struggle, merged into angiotensin; yet, despite these names, it

is likely that the impressive potency of this peptide as a pressor agent may be an incidental or subordinate property, of interest in pharmacology and pathology, but of no direct relevance to a possible physiological role, i.e. as the primary regulator of aldosterone secretion (26). Similar misfortune caused oxytocin (uterine contraction) and vasopressin (pressor action) to be named by reference to one of several biological activities (in mammalian species) of pharmacological interest, again the choice not falling on the most likely physiological role (respectively, milk ejection and antidiuresis in mammals, and necessarily other functions in lower vertebrate classes!).

The impracticalities of this type of nomenclature in neurohypophyseal peptides was made apparent by the prediction and eventual identification of an intermediate compound for which a variety of different names were employed (69).

Despite use of expressions such as "migration inhibitory factor(s)" or "MIF's" as opposed to the more common singular form, there is often widespread use of MIF as a descriptive term for a single substance derived by antigen activation of lymphoid cells. This might be tolerable if the presence or appearance of migration inhibitory activity was only observed in very restricted situations (i.e. antigen activation of lymphocytes from animals exhibiting delayed hypersensitivity to that antigen). However, it is known that this is not the case, as several other sources of similar or identical biological activity exist; thus, lymphocytes produce such biologically active material in the absence of antigenic stimulation (6, 14); substantial activity is evident in culture fluid from various lymphoid, and more especially other non-lymphoid cell types, in established cell lines (40, 56); *in vivo* this activity is demonstrable in lymph derived from both allergic and non-allergic inflammatory responses (42, 50); and finally cell migration inhibition is a biological property (albeit with an optimistic activity of 1 molecule per test chamber!) of the purified proteoglycan (circa 300,000 daltons) present in Kurloff cells of the guinea-pig (66). Perhaps it is recognition of this difficulty that has led to the recent appearance of

subscripts (e.g. $\text{MIF}_{\text{OCB-BGG}}$ and MIF_{ConA}) (64) which practice, if adopted for other activities evident in these culture fluids (e.g. mitogenic factor, skin reactive factor, cytotoxins, etc.) could swiftly generate a sufficiently large body of names to occupy entire symposia sessions.

For such reasons, use of a generic name to encompass the range of biological activities (not due to antibody) that are induced *in vitro* by the incubation of lymphoid cells with specific antigen or mitogen is desirable. It remains to be seen whether adoption of a name suggestive of activation of lymphoid cells such as "lymphokine" (131) is to be preferred to an inert alphanumeric notation, as was used for example in the case of Substance P (33) or 48/80 (57). Finally, it should be noted that there is common usage of M.I.F. to refer to biological activity attributed to a "Melanocyte stimulating hormone-inhibiting factor" and this is presumably in no way related to MIF.

4) Characterization

Perhaps the greatest hazard in attributing a biological activity to a corresponding factor is that this can be presumed to imply that some specific substance is indeed responsible for the observed activity, so providing a basis for an expensive and protracted chemical purification programme. This is a justifiable assumption if the biological property is unique or unusual, not being demonstrable with other unrelated tissue extracts, biological fluids or naturally occurring biological materials. Unfortunately, it is more usual to be concerned with a primary biological activity and an attendant bioassay that is not uniquely sensitive to a single compound or class of compounds and, moreover, it is more than likely that other materials known to be active in the bioassay are present in these crude active preparations. This problem has been repeatedly encountered in purification studies of endogenous mediators with potent pharmacological activity and here parallel bioassay is frequently employed for characteriza-

tion and estimation of active material, the assumption being that no two substances can give the same potency ratios on a number of bioassays (initially selected to emphasize differences) (12). By appropriate selection of assay (bioassay or enzyme assay), distinction of antigen-induced migration inhibition activity from similar activity attributable to other materials known or suspected to be present in culture fluids (e.g. complement components, antigen, antibody, immune complexes, enzymes) may be achieved. The ease with which such distinction can be made is a function of both the magnitude of the index of discrimination for the selected assays, together with the precision of the respective assays. The widespread distribution of material causing macrophage migration inhibition considered together with the relative impurity of the active preparations in current use emphasize the need for use of parallel bioassay to characterize this activity. Although it is widely stated that antigen activation of sensitized lymphocytes induces the appearance of a range of biological activities in the culture fluids, there is little data in the literature to indicate which, if any, of these activities is correlated with the migration inhibition effect and so afford potential parallel assays. Unfortunately, therefore, one must acknowledge that the products of lymphocyte activation are, in this respect, inadequately characterized.

An example that emphasizes the need of characterization to precede attempted purification is provided by "epithelial growth factor" (45), whose activity was shown to be closely correlated with proteolytic enzyme levels in the active fractions rather than a particular specific molecule (4). Lymph node permeability factor is another example of particular relevance to Type IV mediators. Using a preparation analogous to guinea-pig LNPF, namely spleen permeability factor (SPF) from the pig or rat, Davies, Hartley & Schild (25) have shown that in this instance biological activity is a property of a range of different proteins present in the preparation; activity being determined by the distribution of amino acid side chains on these proteins rather than by features of the

tertiary structure of a single compound. Hence, after any fractionation of SPF material, all fractions were accompanied by a biological activity approximately corresponding to the protein concentration, so rendering this activity immune to purification. Reports of partial purification (30, 65) should not necessarily be regarded as further evidence substantiating the existence of a specific active agent causing macrophage migration inhibition, for it is possible by fractionation to select and purify a single active constituent from amidst an array of alternative active compounds.

5) *Measurement of migration inhibition*

In establishing the phenomenon of macrophage migration inhibition by specific antigen or lymphoid cell products, it was initially sufficient to be able to demonstrate distinct differences between experimental and control observations. For the purpose of bioassay and for purification studies, it is essential that quantitative assays are available.

The test usually employed for the measurement of this activity necessitates the induction and collection of peritoneal exudate cells or peripheral blood leucocytes; packing these cells into capillary tubes for subsequent incubation in closed culture; followed by measurement of the area of cell migration. Detailed descriptions of these techniques are provided in Bloom & Glade (10). Such prolonged multistep procedures are subject to numerous technical problems and hence to considerable variation within and between assays. Hughes (44) in proposing use of polyethylene tubing in place of glass, provided one of the few detailed attempts to establish the more critical sources of variation for human leucocyte migration. Despite use of cell migration tests for several years, there is, as yet, no commonly accepted technique so that individual laboratories have their own idiosyncracies. As a consequence of this, there exist numerous reports of minor modifications aiming at the simplification of the assay procedure or reduction in the ratio of cells required per observation; but

perhaps of greater potential significance have been the attempts to develop a cell migration assay involving solid phase culture. Clausen (13) has used migration of cells from wells cut in agar plates to measure PPD sensitivity in human peripheral blood leucocytes. This assay has the advantage of technical simplicity in both assembly and assessment, together with minimal requirements for both cells and antigen. Harrington & Stastny (41) have used a solid phase step for siting the cells in the culture chamber, so eliminating the use of a cell container and also giving regular (i.e. circular) migration areas, facilitating migration area measurement.

Selection of the time for measurement of migration areas has been primarily determined by considerations of operator convenience. In the absence of any detailed understanding of the mechanism of this inhibition, it is possible that the inhibition could be due either to a change in the rate of migration, a change in the latency of onset of migration, or, to a reduction in absolute area that might ultimately be achieved. Time lapse studies by Nathan, Karnovsky & David (54) do indeed suggest that inhibition of migration is manifest as a transient early reduction in migration rate that is most evident in the early phase of the response. Arbitrary selection of a single observation time is unlikely to give optimal discrimination and emphasizes the desirability of definition of the time course of these responses to permit a more rational basis for selection of the observation time or times. Measurement of migration area is generally dependent upon projection or photographic recording of an enlarged image of the cell mass. In inhibited cultures, the migrating cells do not form a uniform sheet at the time of observation (up to 24 hours) except perhaps near the perimeter of the cell mass. Definition of this perimeter is rather uncertain, as might be expected with direct illumination at low magnification. The irregular nature of the perimeter (i.e. the fan shape) restricts application of automated or simplified recording of the response and this remains an unwelcome and laborious facet of the assay. Actual migration areas of normally migrating cells and of cells in inhibited

cultures are rarely reported, preference being given to data transformed to a migration index, which is determined by the ratio of the inhibited cultures to the corresponding controls. The precise method used to calculate limits for these indices often remains unspecified and in the absence of original data is impossible to deduce. A popular empirical (but invalid) procedure is to treat the control areas as not being subject to variation. This is a point of more than academic interest as it results in over-optimistic confidence limits and increases the likelihood that observed differences in indices will appear to be significant. For treatment comparison within an experiment, use of ratios is unnecessary (i.e. the denominator is a constant); it is rather for comparison between successive experiments that ratios have been employed, so as to make allowance for the variability of the migration areas of unstimulated cells.

When ratios are used, it would seem sensible to make a valid estimate of the confidence limits. If the migration index is the ratio of two normally distributed variables then the 95 per cent confidence limits are given by Fieller's theorem (36). Should these variables be log normally distributed the calculation of confidence limits is greatly simplified by use of a logarithmic transform. The magnitude of these confidence limits and hence the significance of the difference between migration indices, is determined by the replicate number and error variance for each group of observations. In cell migration studies, triplicates are commonly used and the standard deviation is often quoted as about 10 per cent, in fact Brostoff *et al.* (11) gave this figure as the average coefficient of variation of recipients in a sample of 940 observations. This is comparable with the range 7.0–24.5 per cent observed in this laboratory in a series of 28 experiments (Bray, M.A., personal communication). In light of this it is instructive to plot the upper 95 per cent confidence limits given by Fieller's theorem for a migration index of 0.8 when the standard deviation of the experiment is 10, 15 and 20 per cent (Figure 1). This shows that 4, 6 and 9 replicates of both the inhibited

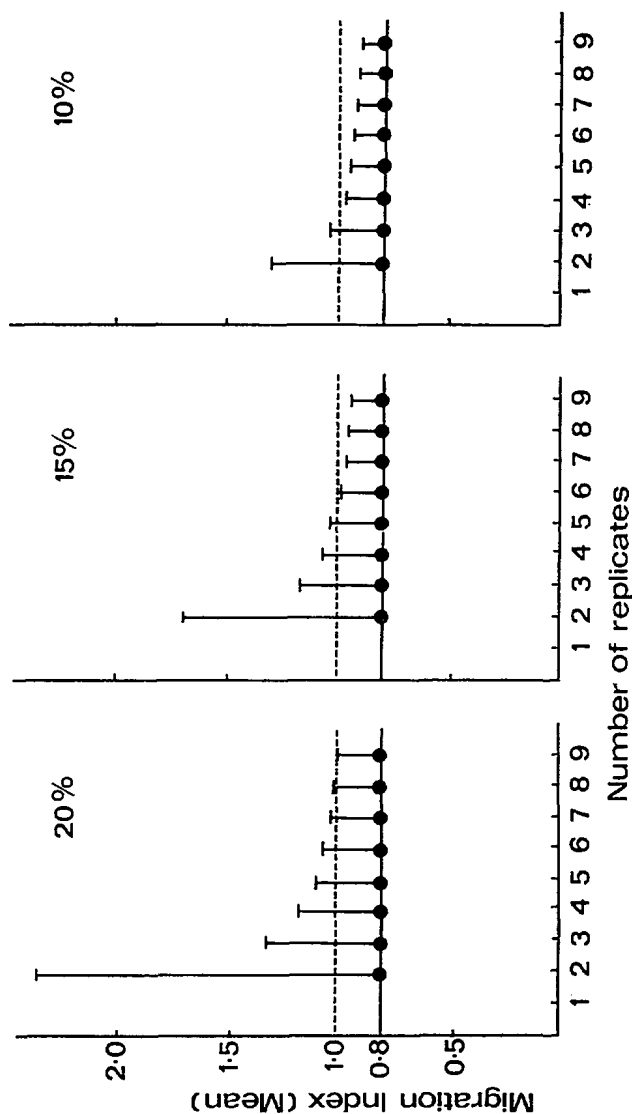


Fig. 1.

Upper 95 per cent confidence limits calculated by Fieller's theorem for a Migration Index of 0.8 (—) with a coefficient of variation of 10, 15 and 20 per cent. For significant difference from unity (---) use of at least 4, 6 and 9 replicates respectively are required.

and control cultures would be required to achieve significance for an index of 0.8. These graphs illustrate the futility of using 0.8 as a fixed level for significance. Rather than selecting an alternative value to 0.8, it is more fruitful to treat each experiment on its own merits (e.g. by estimation of the significance of the difference between mean migration areas following different treatments) and avoid some arbitrary index as indicating significance.

For estimation of migration inhibitory activity in terms of a standard preparation, this ratio becomes redundant; and, since adequate dose-response relationships can be obtained with such inhibitory materials, conventional 2×2 dilution assays may be employed (75). The index of precision that can be achieved with this assay (0.1–0.2) seems acceptable (e.g. in comparison with steroid bioassays (27)), and therefore recourse to less conventional methods of bioassay with their attendant deficiencies may be avoided.

6) *Chemical characterization and purification*

The absence of a precise and specific assay of migration inhibition limits the conclusions that may be drawn from attempts to characterize the activity by chemical manipulation. However, there are a number of relatively clear properties associated with this activity. Thus, the activity preferentially associated with material soluble at 50 per cent saturation with ammonium sulphate (7, 31). It is non-dialysable, and is heat stable at 56° (30 min) but not at 80° (18). Activity is lost following incubation with neuraminidase or chymotrypsin (63, 64). The activity is reported as associated with albumin or pre-albumin, on electrophoresis (65) or column chromatography (30). On the basis of these results, it is generally stated that this activity is associated with a molecular weight range reported variously between 35,000 and 82,000 daltons, with physiochemical properties suggestive of an acidic glycoprotein (7, 30, 63). Migration inhibitory activity in a fraction containing smaller molecular components (circa 12,000 dal-

tons) has been reported, in addition to an active fraction of larger molecular weight (78), but efforts to dissociate a low molecular weight active fraction in the guinea pig proved unsuccessful (30).

TABLE 1

Bovine gamma globulin concentration mg/ml	Plasma albumin extravasation* μlitres	Specific activity units/mg	Apparent purification factor
4.900	71.1	14.5	—
1.255	33.8	26.9	1.9
0.306	27.8	90.8	6.3
0.076	24.1	317.1	21.9
0.0076	13.0	1710.6	118.0

* Plasma albumin accumulation in response to intra-dermal injection of 0.1 ml of BGG solution estimated by use of ^{125}I -albumin (55).

Authors attempting biochemical purification of macrophage migration inhibitory activity acknowledge the limitations imposed by the assay, but often fail to ensure that these limitations restrict their conclusions. The specific activities derived from assays other than at constant activity levels can be biased in favour of high specific activity by deviation of the log dose-response curve from linearity, which is almost certainly the case in this assay. This problem is illustrated by reference to the apparent increase in specific activity of a preparation of bovine γ -globulin (BGG) in an inflammatory assay, when inflammatory activity is determined at successive dilutions of BGG (Table 1). From this data it is apparent that fractionation resulting in substantial loss of material considered together with such bias towards apparent increased specific activity, would combine to favour selection of purification procedures that result in scarcely measurable yields of material with nevertheless substantial apparent purification. Such factors may well contribute to the combination of low yield and high purification factors reported in attempted purifica-

tion of macrophage migration inhibitory activity: 112 μg from 700–900 mg, specific activity increase 1300 (30) and $<0.1 \mu\text{g}$ from 0.64 to 1.92 g, specific activity increase 1600 (65). It seems unlikely that such problems can be circumvented by more novel approaches to purification, e.g. use of paired isotopic markers (72), since the underlying deficiency of an inadequate assay remains unresolved.

7) *Mode of action*

In the original description of antigen-induced cell migration inhibition, macrophage death was clearly evident (67); yet it is likely that this inhibition of cell migration is not dependent upon the cytopathic or cytotoxic potential of these preparations for, although at 24 hours there may be considerable cell death in evidence (37), there are distinctive chemical characteristics associated with cell migration inhibition and cytotoxicity allowing differentiation of these two activities. Thus, the cytotoxic activity, unlike macrophage migration inhibition activity, is susceptible to heating at 60° for 30 minutes and resistant to incubation with neuraminidase (19). Another attendant biological activity possibly influencing cell migration is chemotactic activity and again this may be dissociated from macrophage migration inhibition activity by virtue of differences in electrophoretic mobility, density in caesium chloride gradients and neuraminidase sensitivity (74). It could nonetheless be anticipated that preparations exhibiting cytotoxicity might also exhibit macrophage migration inhibition (39), in this case attributable to a toxic action, which at a gross level cannot be distinguished from active inhibition of migration, and this further emphasizes the need for parallel bioassay in this area of research. Other activities associated with macrophage migration inhibition in culture fluids have yet to be dissociated. It would appear likely that certain activities will prove to be common properties of the same or similar compounds, in particular macrophage activation, macrophage aggregation, and mobilisation of macrophages

within the peritoneal cavity (54, 55). Although these preparations cause inhibition of macrophage migration it has been suggested that they could perhaps better be described as stimulatory rather than inhibitory, since they result, in the longer term, in increased adherence of the macrophage to glass surfaces; more extensive ruffled membrane activity, increased phagocytosis and increased glucose oxidation via the hexose monophosphate shunt (5, 54). The biochemical events underlying these modifications in macrophage behaviour are open to speculation. It would appear that the active material interacts with receptors on the cell surface, since potential reactivity of macrophages can be abolished by their prior treatment with trypsin and since activity can be absorbed out from solution by macrophages (49). The cytochemical changes resulting from this are largely unknown. Agents known to cause elevation of intracellular 3', 5'-cyclic adenosine monophosphate (cAMP) reduce macrophage migration, and this has been taken by Pick (58) to indicate involvement of cAMP as an intracellular mediator of this altered cell behaviour. Conversely, it has been reported that agents expected to increase intracellular cAMP opposed the migration inhibition activity of culture supernatants (46) yet that there is elevation of cAMP levels in human lymphoid cells within minutes of stimulation by PHA (70). It has been further suggested that the ability of colchicine or vinblastine to enhance macrophage migration and to oppose the inhibitory effects of lymphocyte activation products may also be dependent upon involvement of cAMP in microtubule assembly in these cells (59). Since hydrocortisone and dexamethasone are similarly able to oppose the inhibitory effects of lymphocyte activation products (3), it would appear desirable to include direct measurement of cAMP levels to provide more definitive evidence favouring or eliminating this hypothesis.

Whilst little is known of the biochemical sequence of events in macrophage migration inhibition, morphological studies have provided some insight into the process of migration inhibition. Dvorak *et al.* (32) in an ultrastructural study ob-

served extensive cell contact with interdigitating cell membranes in cultures exhibiting antigen-induced inhibition of macrophage migration; and, in parallel with reduced cell motility the glycogen granules associated with pseudopodia of cells from inhibited cultures became relocated into the cell body. These authors demonstrated an extramembranous surface material on the macrophage that is lost or absent in regions of cell contact; the chemical nature of this material is not known, although a glycoprotein composition is suggested. Dvorak *et al.* (32) propose that loss of cell contact inhibition is a feature of macrophage migration inhibition and that this altered cell behaviour is directly dependent upon loss of such surface extracellular material.

8) *Future prospects*

Measurement of cellular immunity is of interest because of the involvement of cellular immunity in reactions to antigens as, for example: in infection; in tumour surveillance; in graft rejection; and in autoimmune phenomena. In these conditions, measurement of cell migration inhibition provides an *in vitro* index of reactivity either directly, by culture of cells with specific antigen; or indirectly, by incubation of cells with specific antigen, any active material so generated being tested subsequently on unsensitized cells. For these tests, it is desirable to combine precision with high sensitivity, features in this situation that are conspicuously absent and seem unlikely to be forthcoming in the near future. One remedy could be to establish some alternative assay based upon measurement of an associated property of these supernatants (e.g. mitogenicity or cytotoxicity) or some other facet of the macrophage response (e.g. cAMP levels or glucose oxidation). Such a course of action necessarily presupposes that these alternative properties of supernatants are indeed quantitatively related to cellular immunity or that such alternative parameters to macrophage migration provide a dose-response relationship of adequate range and sensitivity and can themselves in

fact be measured with greater precision. Whatever outcome such an approach might have, the merits of measurement of cell migration (its relative simplicity and low capital cost coupled with its widespread acceptance) are such that it would appear likely that this method of measuring biological activity will resist early obsolescence. It is therefore probably more relevant to consider what modifications in this assay might be of substantial value and it is suggested that the solid phase techniques of Harrington & Stastny (41) and more particularly Clausen (13) seem likely to supersede or at least co-exist with the capillary tube method.

Progress in purification of this activity is clearly hampered by inadequate amounts of starting material, a difficulty that is compounded by an insensitive and imprecise assay that utilizes considerable quantities of fractionated material. It seems unlikely that the degree of improvement in assay techniques that can reasonably be envisaged will be of critical importance in this area of work and the prospects of highly purified material becoming widely available are not viewed with optimism. However, this cannot excuse the absence of crude but active standard preparations in this area of work. The availability of such material together with reliable information of activity and stability would considerably assist newcomers to this area of research and encourage a more quantitative approach to assays. In this context, in addition to assaying active preparations by reference to a standard preparation, it might be anticipated that reactivity to antigen will more frequently come to be expressed in some conventional manner. Although there are situations in which cells or antigen are in restricted supply, the frequent reports employing antigen at varying dosage for no apparent purpose than to indicate some overall dose-related phenomenon shows that such factors are not limiting and, in this situation, determination of an ED_{50} (estimated dose causing a response 50 per cent of the possible maximum) from the data, could provide a more useful and conventional index of reactivity.

The association of alterations in cell-mediated immunity, in

infectious diseases, in autoimmune disease, in transplant rejection and in tumour induction and rejection allow projection of a rapid expansion of clinical use of this technique; whilst experimentally there is a corresponding interest in animal models of these diseases, as well as in other uses of the technique, e.g. identification of serum factors affecting tumour cell motility (16); determination of reactive sites on antigenic molecules (8) and examination of the biological properties of a highly purified proteoglycan of guinea pig Kurloff cells (66). For such reasons, measurement of cell migration inhibition has become a widely accepted immunological technique, in the space of less than a decade and some indication of the extent of its current and future application is provided by circa 10^3 titles produced by a computer assisted literature search for 1970-72 on this topic. Perhaps the easiest prediction is that this number will be exceeded in the immediate future.

SUMMARY

The background to use of measurement of cell migration as a measure of cellular immunity is outlined. The nomenclature, biological characterization, attempts at purification, methods of measurement and future prospects for use of this technique have been critically evaluated.

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