Disordered function of mononuclear phagocytes in malignant disease

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In previous reports there have been scattered references to possible disorders in function of macrophages in malignant disease. For example, a decrease in macrophage numbers was reported in skin window exudates of patients with Hodgkin's granuloma, while the foreign body inflammatory response within a variety of transplantable tumours in subcutaneous sites in rodents was found to be minimal. More recently there have been reports relating to mononuclear phagocyte functions in cancer. This review considers the evidence for macrophage dysfunction in neoplastic disease both in relation to our own studies and to other recent investigations. The disorders are considered in relation to: (i) cell maturation; (ii) migration and chemotaxis; (iii) phagocytosis, cytotoxicity and related phenomena; (iv) lysozyme secretion; and (v) macrophages within malignant tumours.

Disorders of maturation

Macrophage changes in malignant lymphoma were observed in our laboratory during a series of investigations of the cutaneous inflammatory response. Observations were made on skin window preparations in 20 patients with Hodgkin's disease (all untreated) and 20 with non-Hodgkin's lymphoma (13 untreated), and compared with the findings in 29 normal subjects.

On scanning electron microscopy, the surface appearances of macrophages were generally more varied in lymphoma. Whereas in normal subjects over 90% of the macrophages in two-day skin window preparations showed close-packed surface microvilli, less than 50% of macrophages showed these appearances in similar preparations from lymphoma patients; ridged and ruffled forms were more frequent and on statistical analysis, the difference was highly significant (Table 1). These findings were independent of treatment and no patient was suffering from an infection at the time of study.

The microvillous appearances were typical of mature macrophages, the proportion of cells with this form significantly increasing in normal subjects between the first and second day. The ridged and ruffled appearances, which were similar to those seen in blood monocytes and in monocytes after only a few hours in culture, reflected immaturity.

The increased percentages of such forms in lymphoma patients can be interpreted as a failure in maturation of the mononuclear phagocytic cells migrating from the blood to the site of the inflammation.

This evidence of a maturational defect in patients with malignant lymphoma has been paralleled with observations on the proportion of blood monocytes which mature into macrophages after a week in culture. In these studies, the cells have been assessed by their adhesiveness, cell identity being confirmed by a variety of other standard techniques. Maturation was reported to be depressed in patients with squamous cell carcinoma of the lung, malignant melanoma and renal cell carcinoma. A similar defect was found in patients with breast cancer using a dye elution technique. The applicability of these findings to the in vivo situation is strongly suggested by the fact that depression of maturation could be correlated with overall tumour burden and equated with poor prognosis.

Table 1 Surface features of skin window macrophages in lymphoma

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<th>Day 1 (% ± SD)</th>
<th>Day 2 (% ± SD)</th>
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<tr>
<td>Predominantly microvillus</td>
<td>31.5 ± 20.6** (74.8 ± 15.2)</td>
<td>38.3 ± 19.7** (93.3 ± 5.1)</td>
</tr>
<tr>
<td>Mixed</td>
<td>28.4 ± 13.5</td>
<td>18.7 ± 10.3**</td>
</tr>
<tr>
<td>Predominantly ridged or ruffled</td>
<td>40.1 ± 19.8** (18.5 ± 11.8)</td>
<td>42.9 ± 24.3** (4.2 ± 4.3)</td>
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Corresponding normal values are shown in parentheses. **Indicates a highly significant difference from normal (p<0.01).
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Disorders of migration and chemotaxis

In our skin window studies in malignant lymphoma, there was also a highly significant depression in overall cellularity in both one-day and two-day preparations (Table 2). This was again independent of treatment and affected both Hodgkin’s and non-Hodgkin’s lymphoma patients. These findings may be interpreted as reflecting a defect in mononuclear phagocyte migration. This would also explain earlier reports of a depression in skin window cellularity in both Hodgkin’s disease and non-Hodgkin’s lymphoma. A normal exudate has been reported in Hodgkin’s disease but observations were only made over the first 20 h.

In patients with disseminated carcinomas, significantly fewer macrophages have been found in skin window preparations and in skin chambers. Although these tumours had arisen from a variety of primary sites and the patients had widely differing clinical and therapeutic histories, the overall picture of reduced migration is convincing. In a recent study in which all the patients were untreated, the changes were more marked with advanced disease and extensive nodal involvement, and the depression of the macrophage response could be reversed with immunotherapy or plasma exchange. However, in patients with localised carcinomas, there was an increased monocyte mobilisation which returned to normal when the tumour was resected.

The migration of macrophages into inflammatory exudates has also been studied in rats with subcutaneous transplants of chemically-induced tumours or with intramuscular transplants of syngeneic sarcomas. The number of macrophages elicited in a peritoneal exudate in response to an inflammatory stimulus was decreased. The decrease was related to progressive tumour growth and in advanced disease, there was a complete block in the rats’ capacity to mobilise these cells.

Depression of the chemotactic responses of blood monocytes in vitro has also been reported in malignant disease by numerous investigators. In these studies, the monocyte response to standard chemotactic factors was examined in Boyden chambers. The malignancies included melanoma, carcinoma, lymphoma, sarcoma, and multiple myeloma and in all cases, a significant proportion of patients showed a depressed response. This was related to the severity of the disease and the prognosis and was partially reversed by treatment or immunotherapy.

Disorders in phagocytosis, cytotoxicity and related phenomena

Reports relating to the phagocytic and cytotoxic activities of mononuclear phagocytes in malignant disease have often seemed conflicting. These functions have sometimes been enhanced but in other situations have been normal or depressed. It may therefore be helpful at this point to note that differences may be reconciled if the mononuclear phagocyte response is seen as reflecting a balance between stimulatory and inhibitory influences in the subject concerned. The balance might be expected to be different at different stages of the disease. Infections can also disturb these functions and intercurrent infections, though not specifically mentioned in the literature, may sometimes have complicated the picture.

Phagocytic activity has been studied in vitro by measuring the uptake of micro-organisms, erythrocytes or latex particles. Increased phagocytic activity has been found in blood monocytes and monocyte-derived macrophages in untreated patients with Hodgkin’s disease and miscellaneous carcinomas. However decreased phagocytic activity has also been reported in Hodgkin’s disease, notably in stages III and IV. In skin window macrophages of lymphoma patients, depressed phagocytosis was independent of treatment.

Blood mononuclear cells showed an increased phagocytosis-related reduction of NBT (nitro blue tetrazolium) in micrometastatic melanoma, whereas there was an impairment of NBT reduction in disseminated melanoma. Such observations might suggest that monocytes from patients with minimal disease are in some way activated, in contrast to their functional impairment in more advanced disease. However, the picture is by no means clear-cut. Increased chemiluminescence on phagocytosis was noted in the monocytes of patients with lymphomas, but no difference was found in patients with solid tumours. Similarly, differing effects have emerged from studies of whole-body reticuloendothelial activity. As judged by the clearance of labelled aggregated albumin or lipid test emulsion from the blood, increased activity was found in patients with carcinomas and lymphomas, most of whom were untreated. In studies in mice, bearing subcutaneously inoculated Lewis lung carcinoma, clearance of carbon was initially depressed during tumour growth, then became enhanced, but progressively decreased in the later stages.
In studies of the activity of mononuclear phagocytes against target cells and micro-organisms, varying effects have again been noted. Enhanced haemolytic activity was reported in blood mononuclear cells in malignant melanoma but was similar to normal in a series of lymphoma patients. However, impaired cytotoxicity to target cells was present in other patients with untreated Hodgkin's disease. Colon carcinomas were also sensitised by antibody against reticuloendothelial cells in vitro and against lymphomas against malignant melanoma. Impaired activities were noted in untreated patients with lymphoma but in other studies, such patients showed enhanced staphylocidal and candidacidal activity, a modest depression being noted in successfully treated patients. Bacterial killing was normal in patients with mycosis fungoides.

Fc receptor expression on mononuclear phagocytes also appears to be affected in different degrees. This has been investigated by erythrocyte rosetting techniques. In one series of patients with untreated carcinomas, blood monocytes showed increased Fc receptor expression, but no change was noted in another series. Increased numbers of Fc and C3 receptor sites were displayed by monocytes of cancer patients after immunotherapy. While Fc receptor expression was increased in the blood monocytes of patients with lung cancer, it was locally decreased on the pulmonary alveolar macrophages.

Although no consistent pattern emerges from the above observations on phagocytic and cytotoxic activities of mononuclear phagocytes in malignant disease, they would give general support to a working hypothesis that the frequently reported enhancements are mainly a host reaction which would be expected to predominate where there is a smaller tumour load and a less aggressive condition. Conversely, decreased function would result from activity of the tumour and would be expected to be more marked in advanced disease and in the vicinity of the growth.

**DISORDERS OF LYSOZYME SECRETION**

There is some evidence that the synthesis and release of lysozyme by mononuclear phagocytes are disturbed in malignant disease. Raised serum lysozyme activities have frequently been reported in patients with neoplastic disorders, but in a large series of untreated patients with malignant melanoma, hypernephroma and breast carcinoma (who were free from infections and unrelated gross pathology), raised activities were confined to the ones with localised disease, although they were also found as a transient postoperative phenomenon in recurrent or metastatic colorectal cancer. Plasma activities were raised in a series of patients with untreated Hodgkin's disease; the lysozyme activities reflected the tumour mass and activity since they could be correlated with clinical stage and symptoms, and decreased with cytotoxic treatment. In rats with intramuscular implants of a chemically induced fibrosarcoma, the raised serum lysozyme activity reflected the macrophage content of the tumour and draining lymph nodes. In lymph node macrophages themselves, marked differences were found in the immunohistochemical reaction for lysozyme in untreated Hodgkin's disease. Enhanced lysozyme secretory activity was associated with more favourable clinical and histopathological features, whereas depressed activity was present in patients with poorer prognosis.

From the above observations, it would appear that lysozyme secretion may be affected by host and tumour influences in the same way as the other functions discussed.

**DISORDERS OF MACROPHAGES WITHIN MALIGNANT TUMOURS**

There is abundant evidence for the presence of macrophages within malignant tumours. Observations in experimental murine carcinomas and sarcomas indicated that Fc receptor-bearing cells were present in the tumours and that many if not all were macrophages of host origin. The question arises as to what effect these macrophages exert on the tumour and why it is not eliminated.

In hamsters with transplantable lymphomas, macrophages present in non-metastasising tumours were of large size and on electron microscopy could be seen to be engaged in phagocytosis and disruption of malignant cells, whereas macrophages in metastasising tumours were small and without phagosomes. In transplantable sarcomas in rats, both metastasising and non-metastasising in character, macrophage precursors were present which were apparently inhibited from proliferating by the presence of the neoplastic cells but did so when separated from them in vitro. Similarly macrophage monolayers derived from certain solid tumours in rats were cytotoxic to tumour cells in vitro, although apparently not in vivo.

Evidence that the macrophages found within tumours are functionally depressed was also provided by the report that mice carrying sarcomas or mastocytomas in the footpad were incapable of eliminating *Listeria monocytogenes* from the tumour, although the organism was efficiently eliminated from other sites. The establishment and growth of the tumour may in fact depend on the subversion of mononuclear phagocyte function: macrophages isolated from progressive lesions after the inoculation of Moloney sarcoma cells in mice.
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were less cytotoxic to target cells than those from regressing lesions. However, such subversion is potentially reversible and regression of subcutaneous tumour nodules in patients with malignant melanoma or carcinoma of the breast has been observed after injection of a reticulouendothelial activator (Glucan) directly into the lesion; the regression was associated with the finding of large macrophages with foamy cytoplasm.

The possible prognostic significance of the disorders of macrophages within the tumour may also be important in relation to its progression. The possible prognostic significance of the degree of lymphoreticular infiltration of human tumours was pointed out in a review of the earlier literature. Similarly in a series of autochthonous and early passed murine sarcomas, the tumours with the lowest proportion of macrophages (identified by surface markers and phagocytic property) appeared earlier and killed the host more rapidly. Both human and animal studies have consistently found that fewer macrophages are present in metastasising tumours and increased potential for metastasis was produced in transplanted murine fibrosarcomas when the inoculum was artificially depleted of macrophages.

Thus, it would appear that the natural history of a tumour, including its ability to metastasise, may be influenced both by the number of macrophages gaining access to the tumour mass, and by the extent to which their functional activities have been subverted. However, other factors including the release of soluble tumour-specific antigens may also be implicated in metastatic spread.

Possible mechanisms of disturbed function

The disorders of macrophage function considered above may be largely determined by host and tumour factors of a chemical or physicochemical nature.

Raised concentrations of chemotactic factor inactivator, a naturally occurring regulator of the inflammatory reaction, were demonstrated in the serum of patients with Hodgkin's disease. Similarly, a factor, which is present in the plasma of normal individuals and which inhibits migration of macrophages, was shown to be significantly raised in 70% of patients with untreated solid tissue neoplasms and lymphomas; this factor had a relative molecular mass (rmm) of over 600 000 and was possibly an \( \alpha \) macroglobulin. An inhibitor of macrophage accumulation in vivo and of chemotaxis in vitro was described in the supernatants of murine tumour cells; it had a low rmm (6000–10 000) and was heat-stable. This macrophage chemotactic inhibitor (MCI) enhanced growth of several transplanted murine tumours in vivo, possibly by retarding the localisation of macrophages in the developing tumours. Decreased production of MCI was found in an attenuated strain of murine lymphoma, which behaved like a virulent strain when MCI was concurrently injected. Tumour extracts and sera from mice with Lewis lung carcinoma have similarly been shown to depress macrophage migration in vitro.

In other studies in which chemotaxis of macrophages in vitro was inhibited by culture supernatants, the inhibitory substance apparently bound onto the macrophage cell surface. The presence of a growing tumour (following inoculation of transplantable tumour cells in rodents) also interfered with macrophage accumulation in vivo but required a relatively large threshold number of tumour cells.

Culture supernatants from murine tumour cells (as well as from normal thymus and lymph node cells) have similarly been found to suppress macrophage spreading in culture apparently by inhibiting the fixation of bradykinin to macrophage membranes. A factor of low rmm obtained from various transplantable mouse tumours (both spontaneous and induced) inhibited the ability of cultured macrophages to attach to the substratum and spread.

There is evidence that similar factors may be responsible for the disordered phagocytosis and cytotoxicity. A factor obtained from the supernatants of human colon carcinoma cells inhibited phagocytosis in rabbit peritoneal macrophages and likewise, an antiphagocytic action was shown by supernatants of mouse sarcoma cells, a heat-stable factor with an rmm of over 10 000 being involved. A factor with an rmm of less than 12 000 was liberated into the circulation of mice in the early stages after tumour cell injection and resulted in a short-lived but severe impairment of antibacterial function of mononuclear phagocytes.

The activity of both enhancing and inhibiting factors was indicated by observations on Fc receptor expression on mononuclear phagocytes in vitro. Inhibition was brought about by a heat-stable low rmm factor in normal serum and in supernatants of explants of human carcinomas, although it was not established whether the inhibitory factor was newly produced or merely sequestered in the tumour mass. Enhancement was produced by the serum from patients with untreated solid tumours.

In relation to disordered macrophage maturation, inhibitory activity has been found in the serum of patients with breast cancer, particularly in those with node involvement.

While these various factors of host and tumour origin may be thought of as acting specifically upon
the mononuclear phagocytes to affect their functions, some of the observed changes in malignant disease may well be secondary to the response of lymphocytes and other cells. Lymphokines have, for example, been implicated in inducing cytotoxic activity in macrophages, while evidence has been found to suggest that defective monocyte chemotaxis in mycosis fungoides is secondary to a lack of essential helper lymphocyte function. Non-specific blocking factors which can be removed by plasma exchange could also be implicated.

Summary and conclusions

A complex pattern of disturbed mononuclear phagocyte function is found in malignant disease; in some circumstances, functions appear to be enhanced and in others they are depressed. Enhancement of function may be interpreted as mainly a host response, and macrophage activation may be seen as an expression of an immunological reaction to the tumour, exerted through lymphokines and other host factors. Generalised functional enhancement is a prominent feature in the early stages of neoplastic growth, where there is minimal disease and the tumour is localised. Depressed function reflects subversion of macrophage activity and its presence at the local level from an early stage may be important in the establishment of the neoplasm; in the later stages of the disease, the depression of mononuclear phagocyte functions becomes generalised. The mechanisms of such subversion include soluble factors released by the tumour cells (or locally concentrated normal factors).

Interpretation is complicated by the interaction of factors, with the result that different functions of mononuclear phagocytes in malignant disease can be affected to a different degree in the same patient. Perhaps in some circumstances, mononuclear phagocytes may become "prematurely activated" and thus be prevented from ever attaining the full competence of mature tissue macrophages. Such disturbances of macrophage function are likely to be of particular significance in relation to the body's ability to eliminate tumours.

The interplay of factors of host and tumour origin upon the functions of the mononuclear phagocyte system may not only explain apparent conflicts between findings in different investigations but may also underlie the different rates of growth in different tumours. In the latter respect, the number and functional activity of macrophages gaining access to the neoplastic tissue may be of particular importance. The balance of factors may influence the overall clinical course of the disease, including the likelihood of metastasis and the susceptibility to intercurrent infection.

The importance of investigating further measures to restore and enhance mononuclear phagocyte function in malignant disease is clearly indicated.

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