Immunology 1981 **43** 197

Species restriction in cytostatic activity of human and murine monocytes and macrophages

NANCY HOGG & FRANCES R. BALKWILL* *Imperial Cancer Research Fund Tumour Immunology Unit, Department of Zoology, University College London, London and *Imperial Cancer Research Fund Laboratories, Lincoln's Inn Fields, London*

Accepted for publication 20 November 1980

Summary. The pattern of species restriction in cytostatic activity of human breast-milk macrophages (Mph) and murine starch-activated peritoneal exudate (PE) Mph was investigated. Human Mph had appreciable cytostatic activity only for human target cells and not for murine or avian target cells. In contrast, murine Mph were particularly cytostatic for target cells from heterologous species and not as cytostatic for other murine cells. This difference in the activity of murine Mph was more notable when freshly explanted fibroblasts were used as target cells than when the cytostasis of long-term tissue culture lines was measured. Experiments with peripheral blood monocytes from the two species indicated that this pattern of reactivity may be common to mononuclear phagocytes from other sources. Therefore, human Mph are preferentially cytostatic for target cells of self species; whereas, murine Mph are equally if not more cytostatic for target cells from other species.

INTRODUCTION

There is interest in the role that macrophages (Mph) may play in tumour rejection and suppression (Fink, 1976; Adams & Snyderman, 1979). The work on rodent Mph suggests that this cell type is important in controlling tumour growth but less is known about the capabilities of human Mph (Eccles & Alexander, 1974; Fidler, 1976; Russell & McIntosh, 1977; den Otter, Dullens, van Loveren & Pels, 1977). The process by which Mph directly affect tumour cells involves either cell to cell contact or very close proximity between effector Mph and target cells (Balkwill & Hogg, 1979) and proceeds without the assistance of readily detectable antibody (Hogg & Parish, 1980). This requirement for cell contact would suggest that Mph recognize a specific antigen(s) on the membranes of cells susceptible to Mph. At present nothing is known about the nature of these membrane structures. When cells, which are thought to be Mph, interact with lymphocytes in an immune response to antigen, they do so via the I region molecules coded for by the major histocompatibility complex, MHC, (Feldmann, Rosenthal & Erb, 1979).

The Mph cytostasis assay is an *in vitro* system which can be used for looking at the patterns of Mph recognition. The susceptibility to cytostasis of a variety of cells in tissue culture has been recognized by several workers, but there is very little information concerning the range of cells sensitive to this phenomenon. Some experiments have shown that murine starch-activated peritoneal exudate (PE) Mph, either normal or activated, were more cytostatic towards heterologous than to homologous target cells (Cabilly & Gallily, 1977; Goldman & Hogg, 1978); whereas others showed no difference between the susceptibility of different species target cells (Tagliabue, Mantovani, Kilgallen, Herberman & McCoy, 1979). The cytostatic capacity of human Mph from breast milk (Balkwill & Hogg, 1979), cantharidin blisters (Chang & Yao, 1979) and peripheral blood monocytes (Rinehart, Lange, Gormus & Kaplan, 1978; Mantovani, Jerrells,

Correspondence: Dr N. Hogg, ICRF Human Tumour Immunology Group, University College Hospital Medical School, University Street, London WC1E 6JJ.

0019-2805/81/0500-0197$02.00

Dean & Herberman, 1979a; 1979b) against a variety of human lines has been documented, but their activity against cells of other species has not been thoroughly investigated.

We have examined the patterns of species restriction in the cytostatic activity of Mph from two species, namely human breast-milk Mph and murine PE Mph. The activity of these tissue Mph was compared to that of human and murine peripheral blood monocytes. As targets we have used a range of human, murine and avian cell lines as well as freshly explanted embryo cells from these three species.

MATERIALS AND METHODS

Human breast-milk macrophages

Milk was obtained by manual expression and by breast pump during the early stages of lactation (3–11 days *post partum*). The Mph were isolated by adherence for 30 mins at 37° to petri dishes coated with microexudate (ME) from BHK cells (Ackerman & Douglas, 1978) and characterised as previously reported (Balkwill & Hogg, 1979).

Mouse peritoneal macrophages

Six- to eight-week-old male BALB/c mice were obtained from the animal breeding unit at the Imperial Cancer Research Fund (ICRF) London. Peritoneal exudates were induced by intraperitoneal injection of 2 ml of 2% boiled and autoclaved potato starch (Hopkins and Williams, London) and Mph were isolated from the exudates by adherence to ME plates for 60 mins at 37° (Ackerman & Douglas, 1978) and characterized as previously reported (Hogg & Parish, 1980).

Human and murine monocytes

Human peripheral blood mononuclear cells (PBM) were obtained from normal donors and murine PBM from BALB/c mice by Ficoll Hypaque density ($\rho = 1{\cdot}09$) centrifugation at room temperature. Monocytes were separated from PBM by adherence to ME plates at 37° for 30 mins in the case of human cells and 60 mins for murine cells. After removing non-adherent cells from the ME plates by washing, monocytes were recovered by incubation with 3 mM EDTA. The resulting cells were >99% monocytes as assessed by generalised cytoplasmic staining for α-naphthyl acetate (non-specific) esterase (NSE) enzyme (Yam, Li & Crosby, 1971).

The human and mouse embryo fibroblasts were maintained in RPMI 1640 plus 10% FCS and the avian embryo fibroblasts were maintained in RPMI 1640 plus 1% FCS, 1% chick serum and 5% tryptose phosphate broth obtained from ICRF. In all the reported experiments, the embryo fibroblasts (HuEF, BaEF, WME and ChEF) were used within 1 week of being explanted to tissue culture.

Cytostasis assay

The cytostatic assay was carried out as previously described (Goldman & Hogg, 1978). Briefly, human Mph at appropriate dilutions for the cytostatic assay were cultured overnight in 0·2 ml of DEM 5% FCS in flat-bottomed 96-cell microtest Limbro plates. The preincubation of human Mph was done for convenience. Mph which were used in cytostatic assays on the same day that they were isolated from milk gave similar results to Mph left overnight. Macrophages were prepared from murine PEC and used in an assay on the same day. Mph were incubated with target cells (5×10^3 cells per well) at effector:target cell ratios of 40:1 to 5:1 for approximately 18 hr in DEM 5% FCS. The cells were washed once and pulsed with 0·5 μCi [^{125}I]UdR (Radiochemical Centre, Amersham) per well for five hr. After further washing, the plates were dried, the individual wells were cut out and counted in a Wallac gamma counter.

The percent cytostasis was calculated as

$$\frac{\text{c.p.m. targets} + \text{Mph}}{\text{c.p.m. targets}} \times 100$$

The incorporation of [^{125}I]UdR by target cells alone ranged from 3–12,000 c.p.m. between different cell lines and did not correlate with the degree of cytostasis observed. Mph alone did not incorporate [^{125}I]UdR above background levels.

Target cells

Cell lines and strains. (a) Human—the HEL23 (Balkwill & Hogg, 1979), MDA 157 (Young, Carllean, Mackay & Reeves, 1974) and Chang liver cell lines were maintained as monolayer cultures in RPMI 1640 medium containing 10% fresh foetal calf serum, FCS, (Gibco Europe Ltd, Glasgow); (b) mouse—the NIH-3T3 (N3T3) and BALB/3T3 clone A31 fibroblast cell lines and Kirsten sarcoma virus transformed non-producer cell line, K31 (Aaronson & Weaver, 1971) were maintained as monolayer cultures in Dulbecco's modified Eagle's medium containing 10% fresh FCS (DEM 10% FCS); (c) bird—long-term fibroblast cultures were derived from embryos of

Spafas White Leghorn chickens (SP100), Japanese quail (Q328), Muscovy duck (D800), Golden pheasant (Gph) and L15.B inbred chickens (L151625). These cells were obtained from Dr Robin Weiss, Viral Oncology Laboratory, ICRF and used between passages 3–7 in tissue culture.

Embryo fibroblasts. (a) Man—human embryo fibroblasts (HuEF) were prepared from foetal limb material obtained from vacuum abortions of approximately 10–12 weeks gestation. Tissues were finely chopped with scissors and the resulting pieces incubated in RPMI containing 10% FCS. Fibroblasts grew from the chopped tissue pieces within several days. (b) Mouse—primary cultures of BALB/c (BaEF) and Theilor's Original (WME) embryo fibroblasts were obtained from the ICRF Department of Cell Production. They were prepared by trypsinization from 14-day-old embryos. (c) Bird—chicken embryo fibroblasts (ChEF) were obtained from the ICRF Department of Cell Production or the Tumour Virology Laboratory. They were prepared by trypsinization from 10-day-old embryonated eggs after removal of head and viscera.

A number of control experiments were done in order to ensure that the assay was an adequate measure of Mph-mediated cytostasis. Previously it was shown that macrophage:target cell supernatants collected after a 16 hr co-incubation period did not inhibit target cell uptake of [^{125}I]UdR when added to target cells for a further 16 hr or during the 5 hr [^{125}I]UdR labelling period (Balkwill & Hogg, 1979). Furthermore, contact is required between Mph and susceptible target cell for efficient cytostasis to occur (Balkwill & Hogg, 1979) thus eliminating the need to consider Mph secreted soluble substances that may cause artefacts in some assays of this type. On the other hand, tissue-culture supernatant from ChEF did not cause a significant increase in the uptake of [^{125}I]UdR by human breast-milk Mph (data not shown).

RESULTS

Figure 1 shows the pattern of cytostasis of human breast-milk Mph and murine PE Mph against a range of human, murine and avian cell lines and strains at a ratio of 40:1. The results represent a summary of a series of separate experiments using breast-milk Mph samples from sixteen different women and five experiments with starch induced BALB/c PE Mph. It can be seen from the histograms that the human Mph were more cytostatic for three human targets (mean cytostatic activity for all targets 34·38 ± 16·38% of control

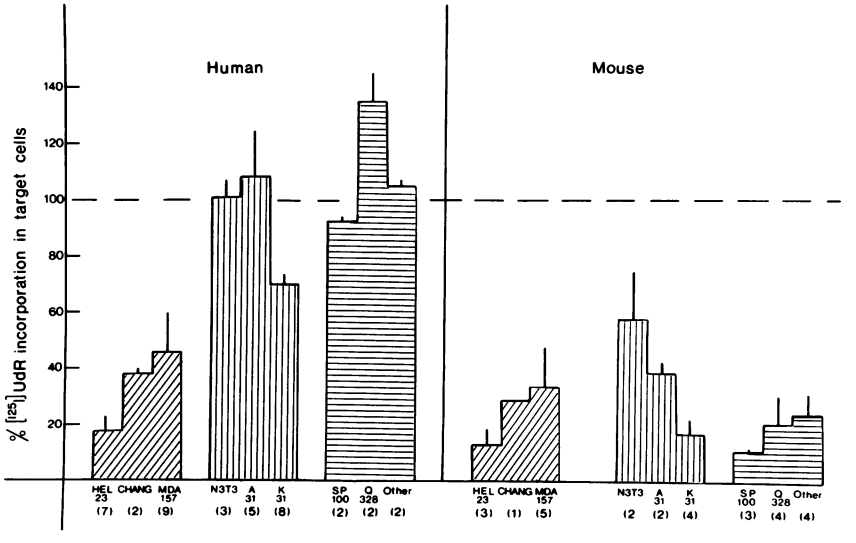

Figure 1. Histogram showing the effect of human breast-milk Mph and mouse peritoneal Mph on the incorporation of [^{125}I]UdR by target cells (100%) after incubation of Mph and target cells at a ratio of 40:1. Human cells were HEL23, CHANG and MDA 157; murine cells were N3T3, A31 and K31 and avian cells were SP100 and Q328. The results from other avian target cells, D800, Gph and L151625, were pooled. The number of experiments carried out with each cell line is indicated in brackets.

target [^{125}I]UdR incorporation) than for the murine targets (mean cytostatic activity for all targets $87 \pm 35{\cdot}10\%$ of control values) or avian targets (mean cytostatic activity for all targets $111 \pm 20{\cdot}7\%$ of control values). The difference between the cytostasis of the human targets was significantly different from the murine or avian targets as assessed by Student's t test (difference between human and murine $P < 0{\cdot}001$, difference between human and avian $P < 0{\cdot}001$). This pattern was consistent for sixteen different individuals. However, two additional individuals were excluded from the compilation of results in Fig. 1 because of the wide deviation from the standard pattern; Mph from one donor showed no cytostatic activity against any target cell and Mph from the other donor were cytostatic for all tested target cells of the three species.

In contrast to the major pattern of species restriction in cytostasis as seen for the human Mph, murine PE Mph were cytostatic for target cells from all three species (mean cytostatic activity for human, murine and avian targets $27{\cdot}22 \pm 13{\cdot}91\%$, $34{\cdot}12 \pm 23{\cdot}23\%$, $20{\cdot}90 \pm 14{\cdot}77\%$, respectively) and there were no significant differences between values for the three groups as assessed by Student's t test.

As differences have been reported in antigen expression between freshly explanted cells and longer-term tissue-culture cells (Thorpe, Parker & Rosenberg, 1977), the cytostatic activity of both the human and mouse macrophages was assessed against freshly explanted embryo fibroblasts, which had been maintained in tissue culture for approximately one week. As can be seen from Fig. 2, which represents data from eight different human Mph samples and four mouse PE Mph samples, the same pattern of reactivity against the species of target cells was observed. Thus the human Mph were more cytostatic for human targets (mean cytostasis $48{\cdot}1 \pm 14{\cdot}43\%$ of control [^{125}I]UdR uptake) than for murine targets (mean cytostasis $84 \pm 8{\cdot}94\%$ of control [^{125}I]UdR uptake) or avian targets (mean cytostasis $105 \pm 11{\cdot}77\%$ of control [^{125}I]UdR uptake). The difference between the cytostasis of the human targets compared to murine or avian were highly significant as assessed by Student's t test (difference between values for human and murine targets $P < 0{\cdot}001$, difference between values for human and avian targets $P < 0{\cdot}001$).

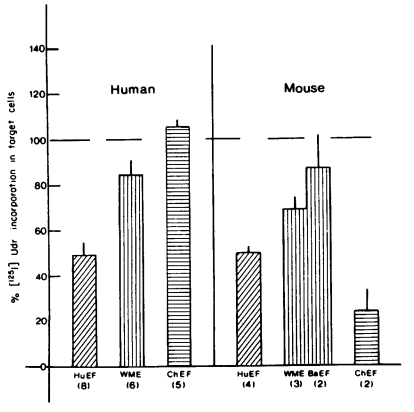

Figure 2. Histogram showing the effect of human breast-milk Mph and mouse peritoneal Mph on the incorporation of [^{125}I]UdR by fibroblast target cells after incubation of Mph and fibroblasts at a ratio of 40:1. The target cells were freshly explanted embryo fibroblasts of either human (HuEF), murine (WME, BaEF) or avian (ChEF) origin. The number of experiments carried out with each type of target cell is indicated in brackets.

The murine PE Mph, on the other hand, reacted against targets from all three species (mean cytostasis—human $50 \pm 7{\cdot}39\%$; avian $24 \pm 24{\cdot}04\%$; murine $75{\cdot}8 \pm 22{\cdot}13\%$ of control [^{125}I]UdR uptake). However, the murine targets were less susceptible to cytostasis than either human or avian targets (difference in values for murine and human targets $P = 0{\cdot}1$; murine and avian targets $P = 0{\cdot}05$. Furthermore, in Fig. 2, there is an indication that between the two types of fresh murine, fibroblast target-cell types, the syngeneic BaEF cells are less susceptible to cytostasis than the allogeneic WME target cells ($87 \pm 25\%$ versus $69 \pm 17\%$ of control [^{125}I]UdR uptake). More extensive experiments would have to be done to establish this point conclusively.

In the experiments which were combined to produce Figs 1 and 2, human Mph were studied in conjunction with mouse Mph in order that differences between them in observed cytostatic activity would not be due to day to day variation in target cell behaviour. In Fig. 3 a typical experiment is presented in which human Mph from a single donor and starch-activated BALB/c Mph were titrated against a panel of human, murine and avian target cells. The pattern of self-species cytostasis for human Mph and cross-species or non-self cytostatic activity of mouse Mph conforms to the data presented in Figs 1 and 2 compiled from many such individual experiments.

In order to see whether the patterns of species restriction in cytostasis were characteristic of other types of macrophages, monocytes isolated from human and murine peripheral blood were examined for cytostatic activity against freshly prepared HuEF, BaEF, and ChEF at an effector:target ratio of 10:1

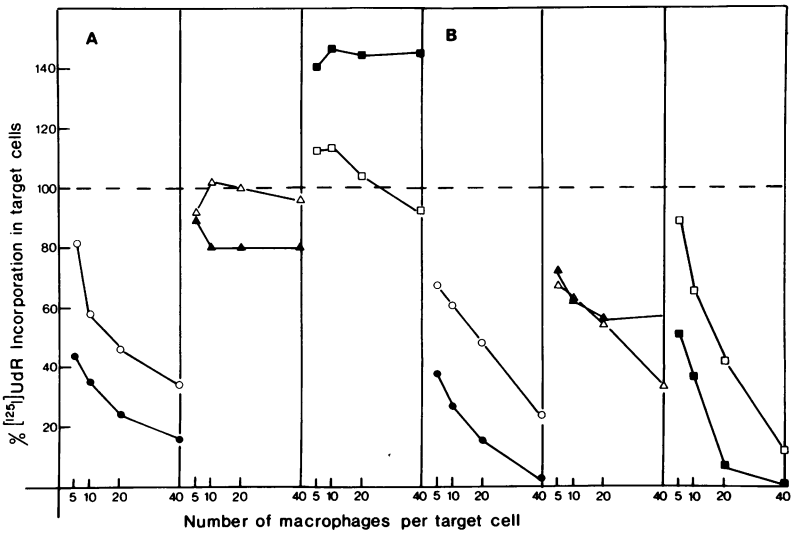

Figure 3. A single experiment showing the [^{125}I]UdR incorporation of human cells, MDA 157 (o—o) and HEL23 p7 (●—●); murine cells N3T3 (▵—▵) and WME p2 (▲—▲); avian cells, SP100 p7 (□—□) and Q238 p6 (■—■) after incubation with human breast-milk Mph from a single donor (A) or BALB/c PE Mph (B) at Mph:target cell ratios of 40:1 to 5:1. The [^{125}I]UdR incorporation c.p.m. ± s.d. of target cells alone (100%) ranged from 3499 ± 581 (WME) to 11,675 ± 593 (MPA 157).

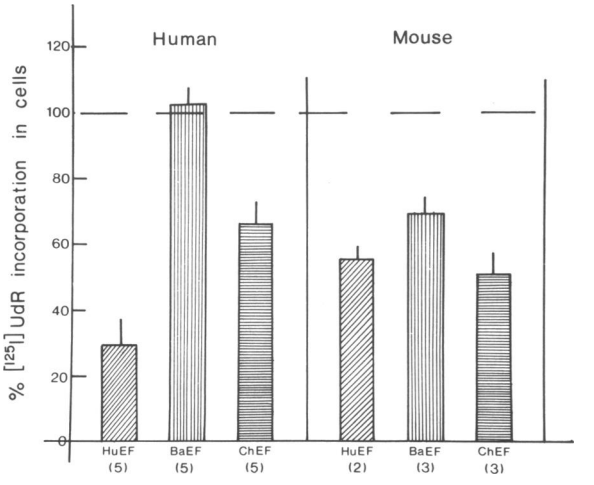

Figure 4. Histogram showing the effect of human and murine monocytes on the incorporation of [^{125}I]UdR by fibroblast target cells after incubation of monocytes and target cells at a ratio of 10:1. The target cells were freshly explanted embryo fibroblasts, HuEF, BaEF and ChEF as in Fig. 2. The number of experiments carried out with each type of target cell is indicated in brackets.

Fig. 4). The combined results of studies on five individuals showed that human monocytes behaved similarly to human breast Mph in that they were more cytostatic for HuEF (mean cytostasis 29·0 ± 9·1% of control [^{125}I]UdR uptake in target cells) than for murine BaEF (mean cytostasis 102·4 ± 5·9% of control [^{125}I]UdR uptake in target cells) or avian ChEF (mean cytostasis 65·4 ± 8·6% of control [^{125}I]UdR uptake in target cells). Differences between the cytostasis of human targets compared to both murine and avian

targets were statistically significant ($P<0.001$) as assessed by Student's t test. The greater cytostasis of avian as compared to murine cells was also significant ($P<0.001$). Mouse monocytes were identical to starch-elicited PE Mph in cytostatic activity towards target cells from the three species (mean cytostasis HuEF $56.0\pm5.6\%$; BaEF $68.7\pm5.5\%$; ChEF $46.3\%\pm7.5\%$ of control ^{125}IUdR uptake). Differences between the three groups were not statistically significant as assessed by Student's t test.

DISCUSSION

The ability to recognize self from non-self has been thought to be an evolutionarily ancient function characteristic of Mph from such diverse creatures as rodents and invertebrates (Burnet, 1974). In this respect human Mph have not previously been examined in any detail. In this paper, the patterns of species restriction in cytostatic activity of human breast-milk Mph and murine starch-activated Mph have been analysed against target cells comprising a wide variety of tissue-culture cells and freshly explanted fibroblasts of human, murine and avian origin. Human Mph were cytostatic for other human cells and, in general, had no cytostatic effect on murine or avian cells. This target cell restriction pattern was consistent for Mph from sixteen diferent breast milk samples. Samples from two other normal donors were not included in the study because they significantly deviated from the general pattern in that Mph from one donor failed to react with any target cells and Mph from the second donor were strongly cytostatic for target cells from all three species. Because of the infrequency of this type of human Mph sample, it is not at present possible to determine the reason for this alteration from the normal cytostatic pattern.

In contrast, murine Mph were particularly cytostatic for non-self target cells, that is, for human and avian cells, and not so effective against other mouse target cells. This is most noticeable in Fig. 2 in which freshly explanted fibroblasts were used as target cells. In addition, there was less cytostasis against syngeneic (BALB/c) fibroblasts than against allogeneic (WME) fibroblasts. This will be more extensively investigated in future experiments. In this context, Somers and Zwilling have reported that BCG-activated murine Mph were more cytostatic for allogeneic than for syngeneic tumour cells (Somers & Zwilling, 1978). It can be noted in Fig. 2 that avian targets are more susceptible than human to mouse Mph. Thus the farther removed phylogenetically a target cell is from a particular mouse strain the more susceptible it is to cytostasis mediated by that type of mouse Mph. In contrast, human macrophages were cytostatic only for allogeneic target cells from self species but not for target cells from other animals.

It could be suggested that the results obtained depended more on the susceptibility to cytostasis of the particular target cells used than on the species recognition capabilities of Mph. The chief argument against this possibility is that susceptibility does conform to a species pattern whether the cells were tumour-derived tissue culture cells (MDA 157, CHANG, K31), 'normal' tissue culture cells (HEL23, A31, N3T3, SP100, Q328, D800 or L15165) or freshly explanted fibroblasts of human, murine or avian origin. These patterns of human and murine Mph cytostasis are clearly demonstrated in the single experiments presented in Fig. 3. It should be noted that the numbers of tumour-derived cells included in these experiments were too small to allow determination of differences between tumour and normal cells in susceptibility to cytostasis *within* a species. Previous work indicates that there is very little difference in susceptibility to Mph-mediated cytostasis of tumour and normal cells adapted to tissue culture (Keller, 1976; Goldman & Hogg, 1978).

Thus, macrophages from man and mouse have contrasting capabilities of discriminating between target cells of self and non-self origin. It is possible that these differences are the result of investigating two different populations of Mph, the human Mph being derived from mechanically- and manually-expressed breast milk and the murine Mph derived from starch-activated peritoneal exudates. The cells responsible for cytostasis in these Mph populations have been shown to possess both Fc and C3 receptors (Hogg & Parish, 1980; Balkwill & Hogg, 1979) but it is possible that the particular target cells for which Mph have specificity depends on the tissue from which the Mph are isolated. For this reason, human and murine monocytes which are the cells of the mononuclear phagocyte series found in peripheral blood, were tested for target cell patterns of cytostasis. These experiments with peripheral blood monocytes indicate that in general, the same particular human and murine patterns of cytostasis apply also to monocytes. It should be noted, however, that human monocytes are inhibitory towards avian fibroblasts (Fig. 4); whereas, the breast milk Mph to a small degree enhance the growth of the

same cells (Fig. 2). This may indicate that subsets of monocytes exist and that the breast milk Mph is derived from a monocyte which fails to interact cytostatically with avian cells. Alternatively, the local environment in which a monocyte finds itself could cause an alteration in its recognition capabilities.

A second type of non-specific killing cell is the NK cell (Herberman & Holden, 1978). It has been more extensively investigated than Mph for cytolytic activity across species barriers. Restriction of cytolysis to target cells of the same species as the NK-cell source has been found in some studies (Nunn, Djeu, Glaser, Lavrin & Herberman, 1976; Shellam & Hogg, 1977) but not in others (Haller, Kiessling, Orn, Karre, Nilsson & Wigzell, 1977) suggesting that subsets of NK cells may exist with different specificities. In one recent report, the cytolytic activity of human NK cells was limited to human cells but mouse and rat NK cells were not species restricted (Nunn & Herberman, 1979). Thus the species differences in human and mouse NK-cell killing appear to be very similar to differences in human and mouse Mph cytostasis reported in this paper.

The ability to discriminate self from non-self tissues is a characteristic of all metazoans (Burnet, 1974). Foreign graft rejection in insects (Lackie, 1979) and snails (Sminia, Borghart-Reinders & Linde, 1974) consists of encapsulation of the graft by Mph-like haemocytes. The more phylogenetically distant the graft, the more rapidly and thickly it is encapsulated by haemocytes. This ability to discriminate between 'degrees' of foreignness is similar to the type of cytostatic activity displayed by mouse Mph described in this paper. Therefore, the pattern of human Mph responsiveness to self versus non-self species appears to differ from the rest of the animal kingdom. It is not certain what the practical value *in vivo* would be of recognizing non-self species. However, benefit would be gained by recognizing that the antigenic display in a particular tissue had been altered from the normal 'self' pattern. In this way, Mph could participate in immuno-surveillance against nascent tumour cells (Adams & Snyderman, 1979) or in controlling normal cell homostasis. Interactions between Mph and autochthonous cells may proceed as a normal event with no ensuing cytostasis; whereas, alterations to varying degrees in the 'recognition molecules' would result in Mph-mediated cytostasis. This present study suggests that murine Mph are able to interact more cytostatically with a broad range of 'non-self' cells than can human Mph. Alternatively, if there are subsets of Mph differing in recognition specificities, then the type of Mph common in most animals may be suppressed in man in favour of a class of Mph perhaps specializing in a narrower range of alterations from 'self'.

ACKNOWLEDGMENTS

We gratefully acknowledge the help of Dr Judy Levi, Department of Obstetrics and Gynaecology, University College Hospital, London, Dr Rosemary E. Gale, Department of Clinical Haematology, University College Hospital Medical School, Professor R. Taylor and the staff at the Obstetrics Unit, St. Thomas's Hospital, London and the staff at Queen Charlotte's and Chelsea Hospital for Women, London.

We thank Mr Ian Todd for his assistance in removal of blood from mice and Drs Robin Weiss and Marc Feldmann for reading the manuscript.

N.H. is supported by the Leukaemia Research Fund of Great Britain.

REFERENCES

AARONSON S.A. & WEAVER C.A. (1971) Characterisation of murine sarcoma virus (KIRSTEN) transformation of mouse and human cells. *J. gen. Virol.* **13,** 245.

ACKERMAN S.K. & DOUGLAS S.D. (1978) Purification of human monocytes on microexudate coated surfaces. *J. Immunol.* **120,** 1372.

ADAMS D.O. & SNYDERMAN R. (1979) Do macrophages destroy nascent tumours? *J. natn. Cancer Inst.* **62,** 1341.

BALKWILL F.R., & HOGG N.M. (1979) Characterisation of human breast milk macrophages cytostatic for human cell lines. *J. Immunol.* **123,** 1451.

BURNET F.M. (1974) Invertebrate precursors to immune responses. *Contemp. Top. Immunobiol.* **4,** 13.

CABILLY S. & GALLILY R. (1977) Studies on the recognition of xenogeneic cells by non-immune macrophages. I. Destruction of chicken fibroblasts in vitro by murine macrophages. *Cell. Immunol.* **29,** 54.

CHANG Y.-H. & YAO C.-S. (1979) Investigation on human macrophage. II. The *in vitro* cytotoxicity of macrophage. *Europ. J. Immunol.* **9,** 521.

ECCLES S.A. & ALEXANDER P. (1974) Macrophage content of tumours in relation to metastatic spread and host immune reaction. *Nature (Lond.)*, **250,** 667.

FELDMANN M., ROSENTHAL A. & ERB P. (1979) Macrophage–lymphocyte interactions in immune induction. *Int. Rev. Cytol.* **60,** 149.

FIDLER I.J. (1976) Macrophage deficiency in tumour bearing animals: control of metastasis with macrophages activated *in vitro*. In: *The Macrophage in Neoplasia* (Ed. by M.A. Fink), p. 245. Academic Press, New York.

FINK M.A. (Ed) (1976) *The Macrophage in Neoplasia.* Academic Press, New York.

GOLDMAN R. & HOGG N.M. (1978) Enhanced susceptibility of virus-infected fibroblasts to cytostasis mediated by peritoneal exudate cells. *J. Immunol.* **121,** 1651.

HALLER O., KIESSLING R., ORN A., KARRE K., NILSSON K. & WIGZELL H. (1977) Natural cytotoxicity to human leukaemia mediated by mouse non-T cells. *Int. J. Cancer,* **20,** 93.

HERBERMAN H.T. (1978) Natural cell mediated immunity. *Adv. Cancer Res.* **27,** 305.

HOGG N. & PARISH C.R. (1980) Surface antigens of the murine cytostatic peritoneal macrophage. *Immunology,* **41,** 187.

KELLER R. (1976) Susceptibility of normal and transformed cell lines to cytostatic and cytocidal effects exerted by macrophages. *J. natn. Cancer Inst.* **56,** 369.

LACKIE A.M. (1979) Cellular recognition of foreignness in two insect species, the American cockroach and the desert locust. *Immunology,* **36,** 909.

MANTOVANI A., JERRELLS T.R., DEAN J.H. & HERBERMAN R.B. (1979a) Cytolytic and cytostatic activity on tumour cells of circulating human monocytes. *Int. J. Cancer,* **23,** 18.

MANTOVANI A., TAGLIABUE A., DEAN J.H., JERRELLS T.R. & HERBERMAN R.B. (1979b) Cytolytic activity of circulating human monocytes on transformed and untransformed human fibroblasts. *Int. J. Cancer,* **23,** 28.

NUNN M.E., DJEU J.Y., GLASER M., LAVRIN D.H. & HERBERMAN R.B. (1976) Natural cytotoxic reactivity of rat lymphocytes against syngeneic Gross virus induced lymphoma. *J. natn. Cancer Inst.* **56,** 393.

NUNN M.E. & HERBERMAN R.B. (1979) Natural cytotoxicity of mouse, rat and human lymphocytes against heterologous target cells. *J. natn. Cancer Inst.* **62,** 765.

DEN OTTER W., DULLENS H.F.J., VAN LOVEREN H. & PELS E. (1977) Anti-tumour effects of macrophages injected into animals: a review. In: *The Macrophage and Cancer* (Ed. by K. James, B. McBride and A. Stuart), p. 119. University of Edinburgh.

RINEHART J.E., LANGE P., GORMUS B.J. & KAPLAN M.E. (1978) Human monocyte-induced tumour cell cytotoxicity. *Blood,* **52,** 211.

RUSSELL S.W. & MCINTOSH A.T. (1977) Macrophages isolated from regressing Moloney sarcomas are more cytotoxic than those recovered from progressing sarcomas. *Nature (Lond.),* **268,** 69.

SHELLAM G.R. & HOGG N. (1977) Gross virus-induced lymphoma in the rat. IV. Cytotoxic cells in normal rats. *Int. J. Cancer,* **19,** 212.

SOMERS S.D. & ZWILLING B.S. (1978) Role of the murine major histocompatibility complex in macrophage-mediated cytolysis. *J. Immunol.* **121,** 2453.

SMINIA T., BORGHART-REINDERS E. & LINDE A.W. (1974) Encapsulation of foreign materials experimentally introduced into the freshwater snail, *Lymnaea stagnalis. Cell Tissue Res.* **153,** 307.

TAGLIABUE A., MANTOVANI A., KILGALLEN M., HERBERMAN R.B. & MCCOY J.L. (1979) Natural cytotoxicity of mouse monocytes and macrophages. *J. Immunol.* **122,** 2363.

THORPE W.P., PARKER G.A. & ROSENBERG S.A. (1977) Expression of foetal antigens by normal human skin cells grown in tissue culture. *J. Immunol.* **119,** 818.

YAM L.T., LI C.Y. & CROSBY W.H. (1971) Cytochemical identification of monocytes and granulocytes. *Amer. J. clin. Pathol.* **55,** 283.

YOUNG R.K., CAILLEAU R.M., MACKAY B. & REEVES W.J. (1974) Establishment of epithelial cell line MDA-MB-157 from a metastatic pleural effusion of human breast carcinoma. *In Vitro,* **9,** 239.