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Organ-Associated Macrophage Precursor Cells as Effector Cells Against Tumor Targets and Microorganisms

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A. Introduction

In the past 10 years much attention has been paid to the phenomenon of natural resistance as a first line of defense against tumors and microorganisms. Such natural resistance can be attributed to a certain degree to resting and activated macrophages [1, 2]. The most widely discussed natural effector cell has, however, been the so-called natural killer (NK) cell. Based on their morphological features, NK cells could be classified either as medium-sized lymphocytes [3] or as immature macrophage precursor cells. These two cell types are, in fact, morphologically indistinguishable. We previously presented evidence [4–9] that the NK compartment consists, at least in part, of cells in the early differentiation stages of macrophages. Nonadherent and nonphagocytic macrophage precursor cells can be obtained from in vitro liquid cultures of mouse bone marrow. These cells exert NK-like activity against YAC-1 cells, but leave P815 cells totally unaffected [4, 5]. The same cells behave as potent effector cells in ADCC against tumor targets [4]. Along this line the same cell type was isolated from human peripheral blood of healthy donors [10], indicating for the first time that such immature cells of the macrophage lineage reside also outside the bone marrow. In the present short review we summarize our recent data indicating that the same cell type can be isolated from the spleen of normal mice and that it can easily and efficiently be induced into organs like the spleen and liver under recruitment or inflammatory conditions [7–9]. As an extension of the previously described NK-like anti-YAC activity, we were able to attribute to this same cell type strong microbicidal activity against *Candida albicans* and *Leishmania enriettii* [6–9]. Since macrophages as a lineage are represented early in phylogenesis and successfully mediated natural resistance and protection long before the specific immune system evolved, their influence on the evolution and development of the specific immune system could be much more pronounced than evidenced at the present moment.

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B. Materials and Methods

All materials and methods have been described in detail [6–9].

C. Results

Macrophage precursors were isolated either from erythrocyte-depleted splenocytes or from liver nonparenchymal cells, after destruction of the hepatocytes by enzymatic treatment. Spleen cells and liver nonparenchymal cells were filtered through nylon-wool columns in order to eliminate all adherent cells. The eluted cells were then applied to a discontinuous Percoll gradient (a slight modification of the gradient described by Timonen and Saksela [11]). By means of this gradient a cell fraction highly enriched for NK activity, and essentially free of small

Table 1. Effect of separation by means of specific antimacrophage antibody-mediated rosetting on the candidacidal and tumoricidal activity of Percoll fraction 40.8–45.3 derived from normal nonadherent splenocytes as compared to its proliferating ability in the presence or absence of CSF-1

Effector cells	Candidacidal activity[a]	Natural killer activity[b]	[^{3}H]dThd uptake (mean cpm)[c]	
			Without CSF-1[d]	With CSF-1
Fr. 40.8–45.3	42.6	40.2	3328	19730
F4/80$^-$	26.1*	26.4	474	5905
F4/80$^+$	59.4*	42.3	2333	27543.7

[a] Candidacidal activity was determined in a 12-h colony forming unit inhibition assay at the effector: target ratio 10:1.
[b] Natural killer activity is expressed as % specific ^{51}Cr release from radiolabeled YAC-1 target cells at the e:t ratio 25:1 in a 12-h assay.
[c] [^{3}H]dthd (1 μCi/well) was added, after 3 days of cultivation, 18 h before terminating the assay.
[d] L-929 fibroblast-conditioned medium was added at the concentration of 20% as a source of CSF-1.
Data are the means of triplicate samples. Standard errors, usually <3%, have for clarity been omitted.
* $P<0.01$, according to Student's t-test.

Table 2. Effect of separation by means of specific antimacrophage antibody-mediated rosetting on the candidacidal, tumoricidal and CSF-1 dependent proliferating ability of Percoll fraction 40.8–45.3 derived from nonadeherent splenocytes from cyclophosphamide pretreated mice (Cy 150 mg/kg, 12 days before the assay)

Effector cells	Candidacidal activity[a]	Natural killer activity[b]	[^{3}H] dThd uptake (mean cpm)[c]	
			Without CSF-1[d]	With CSF-1
Fr. 40.8–45.3	36.64	38.4	763	61250
F4/80$^-$	15.8*	22.2*	819	15650
F4/80$^+$	52*	40.9	1003	77234

[a] Candidacidal activity was determined in a 12-h colony forming unit inhibition assay at the effector: target ratio 5:1.
[b] Natural killer activity is expressed as % specific ^{51}Cr release from radiolabeled YAC-1 target cells at the e:t ratio 12.5:1 in a 12-h assay.
[c] [^{3}H]dthd (1 μCi/well) was added, after 3 days of cultivation, 18 h before terminating the assay.
[d] L-929 fibroblast-conditioned medium was added at the concentration of 20% as a source of CSF-1.
Data are the means of triplicate samples. Standard errors, usually <3%, have for clarity been omitted.
* $P<0.01$, according to Student's t-test.

lymphocytes and granulocytes was gathered [6, 7]. These cells were further separated by an indirect rosetting technique [7–9] on the basis of the expression of the macrophage specific surface marker F4/80 [12]. The cells sorted by this procedure are further referred to as F4/80 negative and F4/80 positive cells.

Data obtained with normal spleen cells separated as described are summarized in Table 1. Data obtained with cells from spleen under recruitment conditions (mice treated with cyclophosphamide 12 days before the assay [7]) are presented in Table 2, and data concerning inflammatory liver cells (mice treated with MVE-2 3 days before the assay [8]) are documented in Table 3. All tables show NK activity and candidacidal activity of the cells as well as their ability to incorporate ^{3}H-thymidin ([^{3}H]dThd) in response to the macrophage-specific growth factor CSF-1. All F4/80 positive fractions gave rise to large macrophage colonies in soft agar. This fraction obviously consisted

Table 3. Effect of separation by means of specific antimacrophage anti-body-mediated rosetting on the candidacidal, tumoricidal, and CSF-1 dependent proliferating ability of Percoll fraction 40.8–45.3 derived from liver non-parenchimal cells obtained from MVE-2 pretreated mice (MVE-2 25 mg/kg, 3 days before the assay)

Effector cells	Candidacidal activity[a]	Natural killer activity[b]	[^{3}H]dThd uptake (mean cpm)[c]	
			Without CSF-1[d]	With CSF-1
Fr.40.8–45.3	29.44	30.3	2994	219143
F4/80$^-$	6.1*	39.9*	1499	4304
F4/80$^+$	44.2*	24.0	3741	33499

[a] Candidacidal activity was determined in a 12-h colony forming unit inhibition assay at the effector: target ratio 2.5:1.
[b] Natural killer activity is expressed as % specific ^{51}Cr release from radiolabeled YAC-1 target cells at the e:t ratio 12.5:1 in a 12-h assay.
[c] [^{3}H]dthd (1 μCi/well) was added, after 3 days of cultivation, 18 h before terminating the assay.
[d] L-929 fibroblast-conditioned medium was added at the concentration of 20% as a source of CSF-1.
Data are the means of triplicate samples. Standard errors, usually $<3\%$, have for clarity been omitted.
* $P<0.01$, according to Student's t-test.

of cells of the macrophage lineage in various differentiation stages, since the response to CSF-1 was heterogeneous. Some cells matured within 24–48 h to typical macrophages without forming colonies whereas others developed small clusters and soon matured to macrophages. Some of the cells also gave rise to large macrophage colonies in response to CSF-1. No cell death was observed in the F4/80 positive fraction under these culture conditions. When the F4/80 negative fraction was cultivated the same way, no early maturation to macrophages could be scored. Colonies developed with a lag phase of about 3–5 days compared to the F4/80 positive fraction. Thus, precursor cells committed to the macrophage lineage are apparently present in the F4/80 negative fraction. These cells are, however, too immature at the time of separation to be recognized by F4/80.

All three tables show that candidacidal activity and NK-like activity as well as proliferation in response to CSF-1 are attributes of the F4/80 positive fractions. Since the F4/80 positive fraction represents a virtually homogeneous population of cells of the macrophage lineage, these data prove the presence, in both organs, of functionally active macrophage precursors. These cells were characterised as nonadherent and nonphagocytic, possessed (like NK cells) a low buoyant density, expressed macrophage-specific surface antigens, and actively respond to CSF-1 with proliferation. In the spleen this cell type has been defined as a constituent of the normal, noninduced organ.

D. Discussion

NK cells are potent tumoricidal effectors, whose lineage has been a matter of discussion for years. NK cells have been described as lymphocytes, based on their morphology and on their inability to phagocytose and adhere. The surface antigens described up to now did not help in assigning these cells to one or the other lineage, since NK cells share surface antigens with lymphocytes [13–15] as well as with cells of the monocyte/macrophage lineage [16–18] and with granulocytes [16, 17]. Evidence for a T-cell nature of NK cells came mostly from IL-2 dependent cytotoxic T-cell clones which, after long in vitro culture, were found to be able to destroy NK targets [19]. These clones, however, have lost their specificity and most probably are not related to any of the in vivo occurring cell types. Freshly isolated NK cells do not express a T-cell receptor [20]. These data substantiate the view that NK cells and T cells are not closely related. A separate lineage has also been proposed for NK cells [21].

Our data demonstrate that a significant part of the the NK activity under normal as well as under inflammatory conditions is exerted by cells belonging to the macrophage lineage. These cells were positively sorted on the basis of the highly lineage-specific macrophage marker F4/80 and proved to be capable of exerting NK and candidacidal activity and of responding to the lineage-specific factor CSF-1 with proliferation and maturation. The nature of the effectors present in the F4/80 negative fraction is an object of current investigation. Cells in the early stages of macrophage differentiation are present in this fraction, as demonstrated by response to CSF-1 stimulation, but as yet it has not been possible to rule out the contribution of another, unrelated cell type.

Another major implication of our work is the possible in vivo role of macrophage precursors. The presence of this cell type in the spleen and liver of normal mice and its inducibility in large amounts upon inflammation suggest that macrophage precursors can actively contribute in vivo to natural resistance. Data describing the contribution of these cells to the resistance against a systemic *Candida albicans* infection in immunomodulated mice have recently been published [22, 7].

The cells belonging to the macrophage lineage derive, according to a current theory [23], exclusively from the bone marrow compartment. Recent investigations performed in our lab, however, strongly point to the existence of an extramedullary generation of macrophage precursors taking place in the spleen and in the liver. In the light of the unexpectedly widespread importance of these cells the advantage represented by several independent sources able to supply macrophage precursors upon induction is obvious.

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