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REGULAR ARTICLE

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Myelopoiesis in the omentum of normal mice and during abdominal inflammatory processes

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Abstract Coelomic cavities are relatively isolated from the systemic circulation of blood cells. Resident cell populations have a proper phenotype and kinetics, maintaining their steady-state populations and their responsiveness to local inflammatory reactions, in which the number and quality of coelomic cells can be greatly increased and modified. We have addressed the question of whether the increase in cell infiltrate in the inflamed abdominal cavity is sustained by the proliferation of myeloid cells in the omentum, and if so what are the characteristics of the progenitor cells involved and how the omentum controls their proliferation and differentiation. In the omentum under normal conditions and with inflammation due to schistosomal infection we found that pluripotent early myeloid progenitors were capable of giving rise to all the myeloid lineages in clonogenic assays, but not to the totipotent blood stem cells. Besides the major haemopoietins (GM-CSF, M-CSF, G-CSF, IL-5), the omentum stroma constitutively expressed SDF-1α, the chemokine which elicits homing of circulating early haemopoietic progenitors. While normal omentum stroma produced LIF, its expression was substituted by SCF in inflamed tissues. In the first situation a slow steady-state renewal of progenitors is potentially favoured, while their intense expansion may be predominant in the latter one. We propose that the increase in cells in the abdominal cavity in inflammatory reactions is due to the enhanced input and expansion of early myeloid progenitors sustaining the in situ production of abdominal cell populations, rather than to the input of systemic circulating inflammatory cells.

Keywords Omentum · Milky spots · Mesenterium · Schistosomiasis · Myelopoiesis · Mouse (C3H/HeN)

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Introduction

The peritoneal cavity is formed early in embryogenesis, and it remains relatively isolated from the systemic blood cell circulation throughout life. Intraperitoneal resident cells have their own phenotypic characteristics and proliferation patterns, which maintain their steady-state populations, but they may also circulate through the main lymphatics, and reach extraperitoneal sites under normal conditions. In inflammatory reactions of the abdominal cavity, rapid and extensive modulation of the peritoneal cell populations can be due either to the expansion of resident peritoneal cells or to the entry of inflammatory cells from the mesenteric blood and lymphatic vessels (Tsilibary and Wissig 1987; Wassilev et al. 1998).

Under normal conditions, peritoneal cells originate from the mesenteric tissues, mainly from the mesenteric milky spots and from omentum (Carr 1967). These coelom-specific structures have been characterised as the “coelom-associated lymphomyeloid tissue” (CALT) (Lenzi et al. 1996). Omentum is the major abdominal

CALT organ, consisting of two mesothelial layers, a central connective tissue containing fibroblasts and adipocytes with the associated extracellular matrix, in which focal regions with a peculiar cell composition are designated the "omental milky spots" (Ranvier 1870; Beelen et al. 1980a). They are particularly abundant in the omental fat band and are composed of numerous aggregates of macrophages and lymphocytes. Omental milky spots are generally considered to be the origin of peritoneal macrophages, which are the first line of defence in the abdominal cavity (Beelen et al. 1980b; Mandache et al. 1985). Previous morphological and isotope-labelling studies have shown the presence of lineage-restricted myelopoietic precursor cells in situ, and a proliferative activity of resident macrophages in the omentum has been suggested (Aronson and Saahar 1965; Dux 1986). Ultrastructural studies of endogenous peroxidase and the use of specific-stage monoclonal antibodies have shown that precursor cells, such as monocytoblasts, can be found inside the omental milky spots and more differentiated cells are found in the peripheral areas (Beelen et al. 1980b; Wijffels et al. 1992). In addition, megakaryocytopoiesis was reported in NZB mice, and small erythropoietic foci were detected in milky spots of normal mice injected with erythropoietin into the peritoneal cavity, rather suggesting a broad haemopoietic capacity in loco (Takemori et al. 1994; Hirai et al. 1994).

Acute inflammatory reactions in the abdominal cavity elicit mobilisation of circulating cells, through permeabilisation of the small mesenteric blood vessels and cell diapedesis guided by cytotactic stimuli. Moreover, in chronic inflammation, the local production or expansion of the inflammatory cell infiltrates may potentially participate in their maintenance and further increase. We have chosen to study inflammatory reactions of the abdominal cavity in the murine model of schistosomal infection. Human and experimental infections with *Schistosoma mansoni* are characterised by a permanent presence of adult worms in the mesenteric venous system. Each worm-pair deposits several hundred eggs per day in venules of the intestinal wall. The eggs are retained in the subserosal venules, or are washed by the mesenteric and portal blood flow into the liver, where they embolise in the periportal venous radiculi. These eggs induce the formation of periovular granulomatous inflammatory reactions in liver and in other intra-abdominal tissues. Periovular granulomas consist predominantly of macrophages, lymphocytes and eosinophils (Goennert 1955).

In the early phase of schistosomiasis, inflammatory cells are essentially of bone marrow origin, and up to 30–50% of the bone marrow may be involved in the production of eosinophil granulocytes. In the chronic phase, extramedullar myelopoiesis has been described, associated with diffuse or granulomatous inflammatory reactions in tissues attained by parasites, involving the monomacrophagic, eosinophilic and mast cell lineages (Borojevic et al. 1989; El-Cheikh et al. 1991; Brito and Borojevic 1997; Dutra et al. 1997). This peripheral production and differentiation of inflammatory cells is complementary to their systemic production in the bone marrow, allowing the establishment of a relative equilibrium between the host and the parasite in the chronic phase, in which the disease causes only mild or subclinical manifestations (Borojevic 1992).

Previous studies have shown that the peripheral production of inflammatory cells in chronic schistosomiasis may involve also the peritoneal cavity, in which eosinopoiesis, lymphopoiesis and plasmacytogenesis have been reported (El-Cheikh and Borojevic 1990; Weinberg et al. 1992; El-Cheikh et al. 1994), as well as production and subsequent diversification of the phenotype expression in the monomacrophagic cell lineage (Godoy et al. 1989). In the present study, we addressed the question of the involvement of omentum in the production of myeloid cells in the abdominal cavity under normal and inflammatory conditions. We questioned whether this coelomic site of myelopoiesis was independent of the systemic source of blood stem cells, and whether omentum contained the haemopoietic environment required for a long-term production of myeloid cell lineages.

Materials and methods

Animals and schistosome infection

C3H/HeN mice of both sexes were obtained from the colony bred at the Federal University of Rio de Janeiro. One-month-old mice were infected by transcutaneous penetration of 40 *Schistosoma mansoni* cercariae (BH strain, Oswaldo Cruz Institute, Rio de Janeiro, Brazil) through the tail skin. Mice were studied after 45–55 and 90–95 days of infection, corresponding to the acute and chronic phases of disease, respectively (Borojevic et al. 1984). The normal animals included in the study were rigorously age matched.

Growth factors

Human recombinant erythropoietin (rhEPO) was purchased from CILAG, São Paulo, Brazil. Murine recombinant granulocyte-macrophage colony-stimulating factor (GM-CSF), interleukin (IL)-3 and IL-5 were purchased from Sigma Chemical Co., St. Louis, MO. The cell lines MM3, L929 and WeHi 3B were obtained from the Rio de Janeiro Cell Bank (PABCAM, Federal University, RJ, Brazil) and their supernatants were also used as a source of GM-CSF, M-CSF and IL-3, respectively.

Cell preparations

Non-adherent omentum cells and resident cells of the peritoneal cavity were obtained from normal or infected mice. They were killed by ether and the peritoneal cells were harvested under sterile conditions, after the injection of 5 ml Dulbecco's minimum essential medium (DMEM, Sigma), supplemented with 10% foetal bovine serum (FBS) (Cultilab, Campinas, SP, Brazil) into the abdominal cavity. The omenta were aseptically removed from the peritoneal cavity, pooled and submitted to digestion with 1 mg/ml collagenase IA (Sigma) in Dulbecco's medium, at 37°C for 30–45 min, with gentle stirring. The dissociated cells were plated in 25-cm^2 flasks (Nunc, Denmark) in the RPMI 1640 medium (Sigma) at 37°C under 5% CO_2, for 1–2 h. Non-adherent cells were harvested from the supernatant, washed, quantified by counting in a haemocytometer and prepared for further experimentation. The adherent cells were maintained for 4–8 days under the same

Table 1 Primers used for reverse transcription polymerase chain reaction (RT-PCR) analysis for mRNA expression in omentum stromal cells of normal mice, and mice with acute or chronic schistosomiasis

Name	Direction	Sequence	Expected product size (bp)	PCR conditions
β-Actin	Sense Antisense	5′GTGGGCCGCTCTAGGCACCA (A) 3′ 5′CTCTTTGAT GTC ACG CAC GAT TTC 3'	560	40 cycles: 94°C, 30 s, 60°C, 1 min, 72°C, 1 min; 1 cycle: 72°C, 10 min
IL-5	Sense Antisense	5′ ATG ACT GTG CCT CTG TGC CTG GAG C 3′ 5′ CTG TTT TTC CTG GAG TAA ACT GGG G 3′	242	
G-CSF	Sense Antisense	5′CTC AAC TTT CTG CCC AGA GG 3′ 5′ AGC TGG CTT AGG CAC TGT GT 3′	336	
GM-CSF	Sense Antisense	5′AGA AGC TAA CAT GTG TGC AGA CCC G 3′ 5′ATT CCA AGT TCC TGG CTC ATT ACG C 3′	313	
M-CSF	Sense Antisense	5′ TGC CCT TCT TCG ACA TGG CT (G) 3′ 5′ GCA AAC AGG ATC ATC CAG CTG TTC 3′	212	
SCF	Sense Antisense	5′CCG GAT CCT GGA GCT CCA GAA CAG CTA A 3′ 5′ GGC TGC AGT CCA CAA TTA CAC CTC TTG AA 3′	830	
SDF-1α	Sense Antisense	5′ CTT GGT AAT TGT CCC TGA GTC C 3′ 5′ GGC ATT ACT ATG GCT CCA CTT C 3′	343	
LIF	Sense Antisense	5′ AGA GTC CAG CCC ATA ATG AAG GTC 3′ 5′ GGC CTG GAC CAC CAC ACT TAT GAC 3′	621	40 cycles: 94°C, 30 s, 62°C, 1 min, 72°C, 1 min; 1 cycle: 72°C, 10 min

culture conditions, then trypsinised in order to eliminate the trypsin-resistant macrophages, and the purified connective tissue cells were used for further studies as stroma feeder-layers and for the reverse transcriptase-polymerase chain reaction (RT-PCR) analysis of gene expression. Bone marrow cells were obtained by flushing femurs of normal mice with 5 ml DMEM with 10% FBS. Cells were plated in the same medium at 37°C under 5% CO_2, for 1–2 h, and the non-adherent bone marrow cells were harvested and used in clonogenic and co-culture experiments with omentum stroma cells.

Liquid cell cultures

Non-adherent omentum cells from normal and infected mice were plated in 24-well culture plates (Nunc, Roskilde, Denmark) in McCoy 5A medium (Sigma) with 5% FBS. Recombinant murine IL-5 was added to culture medium at 10 ng/ml alone, or supplemented with IL-3 (100 ng/ml) and GM-CSF (100 ng/ml). Cells were harvested after 24, 48 and 72 h, quantified, centrifuged on glass slides in a cytocentrifuge, stained with standard May-Grünwald and Giemsa solutions (MGG) and analysed morphologically. Immature eosinophils with ring-form nuclei were distinguished from mature cells with segmented nuclei. Since eosinophil granulocytes survive for less than 24 h in culture, only cells displaying a normal cell structure were counted, while pyknotic and dead cells were not considered.

Clonogenic assays

Soft-agar cultures were prepared as described by Heyworth and Spooncer (1993). Briefly, non-adherent omentum cells were plated at 5×10^5/ml in double layer soft-agar assays. The bottom layer was prepared at a 0.4% final agar concentration in Iscove's medium (Sigma) with 10% FBS, plated in 35-mm Petri dishes (Nunc). The upper layer containing the cells (0.33% final agar concentration) was supplemented either with 20% supernatants of MM3 cells or L929 cells, or with rGM-CSF at 400 UI/plate. The cultures were done in duplicate or quadruplicate. Alternatively, bone marrow cells were plated over a feeder-layer of adherent omentum cells obtained from normal or infected mice, or adding conditioned medium of these stromal adherent cells. The colonies (>50 cells) and clusters (<50 cells) were quantified after 7 and 12 days of culture under the inverted microscope. Morphological analyses were confirmed in agar cultures fixed overnight with formol vapour, floated onto microscope slides, dried and stained with MGG.

Early erythroid progenitors (BFU-e), pluripotent colony-forming cells (CFU-Mix), and myeloid progenitors were quantified in the methylcellulose cultures (Heyworth and Spooncer 1993). Briefly, non-adherent omentum cells were plated in four-well culture plates (0.25 ml/well) in Iscove's medium containing 0.7% methylcellulose (4000 cps) supplemented with 10^{-5} sodium selenyte, 2 mM L-glutamine, 10^{-3} M α-monothioglycerol, 1% fraction-V deionised bovine serum albumin and 20% FBS. Supernatants of MM3 and WeHi 3B cells, and 2 U/ml rhEPO were added prior to the addition of cells. Non-adherent omentum cells were plated at a final concentration of 10^6 cells/ml and incubated at 37°C in a humidified atmosphere with 5% CO_2. Each assay was set in duplicate. Colonies were counted after 7 and 13 days of culture. For morphological characterisation, individual colonies were picked up from cultures, cytocentrifuged, fixed with methanol and stained with MGG stains.

Dexter-type cultures

Long-term Dexter-type cultures were prepared following Spooncer et al. (1993) with modifications. Briefly, the adherent connective tissue cells from omentum of normal or schistosome-infected mice were used as stroma feeder-layers. Cells were cultured into 25-cm^2 flasks (10 ml/flask) (Nunc), fed weekly with DMEM supplemented with 20% HS, 10 µM hydrocortisone, 100 U/ml penicillin, and 100 µg/ml streptomycin, and maintained at 37°C under 5% CO_2. After the establishment of confluent stroma layer, cultures were inoculated with freshly collected non-adherent bone marrow cells (1×10^6 cells/ml) of normal mice. The cells in the supernatants of co-cultures were harvested weekly, counted in a haemocytometer, cytocentrifuged onto microscope slides and stained with MGG stains.

RNA extraction, cDNA preparation and RT-PCR amplification

The total RNA was isolated from omentum-derived stromal cells of normal or schistosome-infected mice. Cells (5×10^5) were lysed

with Trizol reagent (Gibco Brl, Gaithersburg, MD) and the RNA was extracted following the manufacturer's instructions. The cDNA was prepared with total RNA, using reverse transcriptase (Gibco) following the standard protocol. Genomic DNA contamination was controlled by using mock cDNA samples, in which reverse transcriptase had been omitted. Oligonucleotide primers used for GM-CSF, G-CSF, M-CSF, IL-5, leukocyte inhibition factor (LIF), stem cell factor (SCF), SDF-1α and β-actin PCR amplifications, the expected product size and PCR conditions are listed in Table 1. DNA molecular weight markers were 100-bp DNA ladders from Gibco. PCR products were separated by electrophoresis on 1.5% agarose gels and stained by ethidium bromide.

Statistical analysis

Significance of the results was determined using the Mann-Whitney *U*-test, and differences were considered significant at $P<0.05$.

Results

Cell populations and differentiation potential of precursor cells from the omentum

In the normal mouse omentum, milky spots can be detected macroscopically as small opaque patches. In schistosome-infected mice their number and size increased two- to threefold. The total number of cells obtained after dissociation of omentum with collagenase increased in the acute and even more in the chronic phase of the disease, reflecting the hyperplasia of the organ during infection (Fig. 1). A similar increase was observed for resident cells in the peritoneal cavity. The number of non-adherent cells also increased, representing approximately 50% of total cells. The adherent fraction cells of omentum consisted essentially of connective tissue cells and macrophages, while in the peritoneal cavity they contained only macrophages.

The differential cell counts of non-adherent cells indicated a relatively constant ratio of the two major populations: lymphocytes and monocytes (Table 2). A significant increase in eosinophils was observed in both sites. It was relatively low in the acute phase, when bone marrow and blood eosinophilia are at the highest levels, and strikingly increased in the chronic phase, when the systemic eosinophilia is decreased and when tissue eosinophil proliferation takes over the production of this cell lineage (El-Cheikh et al. 1991; Geuskens et al. 1991). A peripheral increase in eosinophils in tissue inflammatory infiltrates has been interpreted as increased migration, extension of the cell life through protection from apoptosis, or a local expansion of the lineage precursors. In coelomic peritoneal and pleural cavities, we have already shown in situ proliferation of eosinophils (El-Cheikh and Borojevic 1990; Perez et al. 1993). Liquid cultures of non-adherent cells harvested from omentum have shown that the capacity of eosinophil production increased in accordance with the observed increase in eosinophils in situ, indicating that it was due in large part to extramedullar production of this cell lineage (Fig. 2A). We questioned whether the increase in eosinophils in omentum

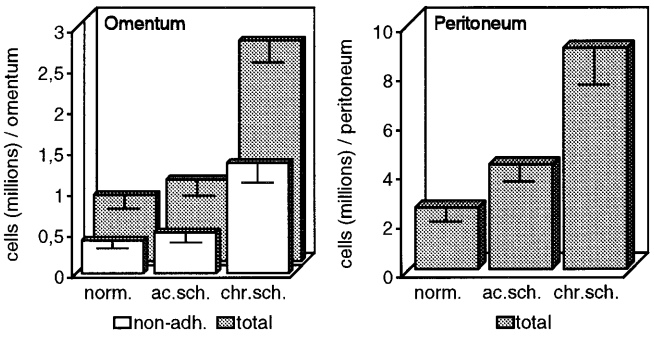

Fig. 1 Total cell numbers and the non-adherent fraction in the omentum and in the peritoneum of normal mice (*norm.*), mice with acute schistosomal infection (*ac.sch.*) and chronic infection (*chr.sch.*). Results represent mean values and standard deviation; $n=48$ for chronic, $n=81$ for acute schistosomiasis, and $n=129$ for normal mice

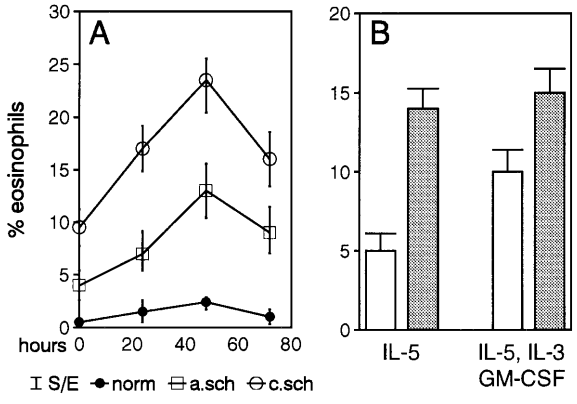

Fig. 2 **A** Percentage of eosinophil granulocytes in liquid cultures of non-adherent omentum cells from normal mice and schistosome infected mice, in the presence of GM-CSF and IL-5 cytokines. Differential counts were done at 0-, 24-, 48- and 72-h intervals. Mean values and standard errors of three independent experiments are shown. **B** Percentage of immature (*open bars*) and mature eosinophils (*full bars*) after 48 h in culture of omentum cells from mice with chronic schistosomiasis, in the presence of IL-5 alone or combined with IL-3 and GM-CSF. *$P<0.05$

Table 2 Differential counts (%) of non-adherent cells from omentum and of peritoneal cells. Relative numbers of cells counted in cytosmears, $n=400$ cells, $n=11$ for normal mice, $n=15$ for acute and $n=20$ for chronic schistosomiasis (*L* lymphocytes, *M* monocytes, *N* neutrophil granulocytes, *Eos* eosinophil granulocytes, *Mast* mast cells)

	L	M	N	Eos	Mast
Omentum					
Normal	40–65	30–53	1–3	0–2	0–2
Acute	45–65	35–55	1–3	4–7	0–2
Chronic	43–60	32–58	2–5	15–20	0–2
Peritoneum					
Normal	40–60	37–45	0–1	0–1	1–3.5
Acute	45–60	42–60	0–2	3–6	1–3.5
Chronic	33–46	37–49	0–2	12–25	0–1.5

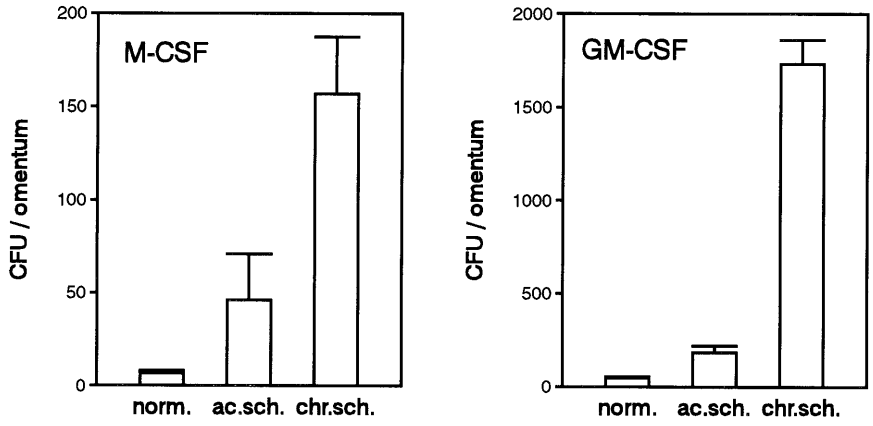

Fig. 3 Numbers of colony-forming units (*CFU*) in soft-agar clonogenic assays of the non-adherent cells obtained from omenta of normal mice (*norm.*) and mice with acute or chronic schistosomal infection (*ac.sch.* and *chr.sch.*) stimulated with M-CSF or GM-CSF. Responses to GM-CSF (colonies + clusters) and to M-CSF (colonies) were analysed at 7 and 12 days, respectively. Results represent mean values and standard errors of three independent experiments. $P<0.05$ for comparison between all the columns

Table 3 Differential counts of colony-forming cells in a non-adherent fraction of omentum cells. Results show mean values of three independent experiments and standard errors. The differential counts (%) show number of colonies containing macrophages (*M*), neutrophil granulocytes (*G*), erythrocytes (*E*), neutrophil granulocytes and macrophages (*GM*) and colonies with several erythroid and myeloid lineages (*Mix*)

CFCs[a]	M	G	E	GM	Mix
Normal	25.2±7.4	5.3±2.6	5.0±1.1	47.5±7.0	17.0±6.8
Acute	12.5±6.6	1.5±0.7	13.0±3.6	18.2±5.4	55.1±9.6
Chronic	32.1±10.4	3.5±1.1	7.6±2.6	12.9±5.6	44.0±7.6

[a] Quantification of CFCs in non-adherent omentum cells from normal mice, and mice with acute or chronic schistosomiasis

and peritoneum was due to terminal expansion of committed progenitors known to be responsive essentially to IL-5, or to expansion of earlier progenitors that respond also to IL-3 and GM-CSF. We observed that the association of these three haemopoietins increased significantly the presence of immature eosinophils, indicating that eosinophil granulocytes differentiated from earlier progenitors (Fig. 2B).

We further addressed the question of the clonogenic potential of myeloid progenitors in the omentum in the soft-agar assay, in the presence of GM-CSF or M-CSF (Fig. 3). For both haemopoietins, the relative increase in such progenitors was much higher in the chronic phase of the disease. GM-CSF induced formation of both clusters and colonies, indicating stimulation of progenitors in different stages of commitment and maturation, while M-CSF induced mostly formation of colonies, reflecting the presence of progenitors already committed to the monomacrophagic cell lineage with a high proliferation capacity. The methylcellulose clonogenic assays were done in order to fully characterise myeloid progenitors in the omentum (Table 3). Besides the colonies containing only macrophages, granulocytes or erythrocytes, we found bipotent and pluripotent progenitors, which produced all the myeloid lineages including megakaryocytes and mast cells. They showed a tenfold increase per omentum in the chronic phase of the disease (Fig. 4, Table 4).

Taken together, these results indicated that the omentum tissue contained a pool of early pluripotent myeloid progenitors, which could produce resident cells in the peritoneal cavity and which were responsive to the increased demands associated with abdominal inflammatory reactions.

Myeloid lineage growth-promoting activity of the omentum stroma cells

Maintenance of the self-renewal capacity of early blood cell progenitors, as well as differentiation of the committed precursors, is known to depend upon haemopoietic environments. The presence of multipotent progenitors in the omentum raised the question of the capacity of the

Table 4 Characterization of the clonogenic capacity of colony-forming cells from omentum (*G* granulocytes, *M* macrophages, *E* erythrocytes, *Mg* megakaryocytes, *Mt* mast cells, *ND* not detected)

	%[a]			Colonies/omentum[b]		
	Normal	Acute	Chronic	Normal	Acute	Chronic
Single cell type	35.5	27.0	43.1	6	15	52
GM	47.5	18.2	12.9	8	12	16
EM	6.8	12.9	12.0	2	7	14
EMg	3.4	5.8	16.0	1	3	20
EMMg	ND	1.5	ND	0	1	0
GEM	3.4	19.6	8.0	1	11	10
GEMMg (Mt)	3.4	15.2	8.0	1	8	10

[a] Results represent differential distributions of colonies obtained in a representative clonogenic assay
[b] Number of colonies/omentum detected in the same experiment

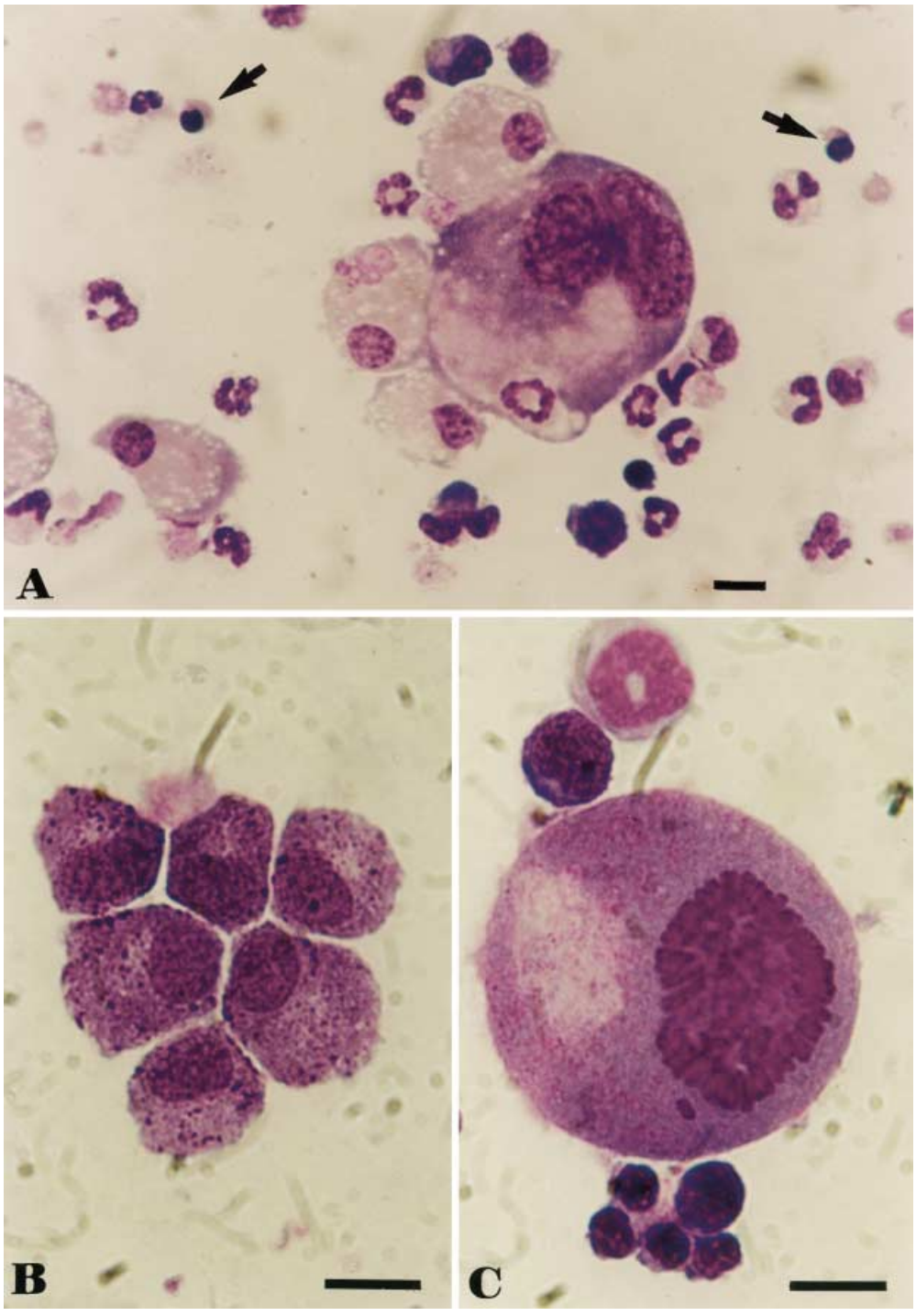

Fig. 4A–C Cytocentrifuge preparations of colonies from methylcellulose clonogenic cultures stained with MGG. **A** A clone derived from a Mix-CFU showing a megakaryocyte, several macrophages, early granulocytes, and erythrocytes extruding their nuclei (*arrows*). **B** A cluster of mast-cell precursors found in a GEMM-CFU. **C** An early megakaryocyte in cell division, with a cluster of erythroblasts and a myeloid cell. *Bar* 10 µm

omentum stroma cells to support proliferation and/or differentiation of myeloid progenitors. Stroma feeder-layers were established from adherent omentum-derived cells of normal and infected mice by serial trypsinisation that eliminated the trypsin-resistant macrophages. Their growth-supportive capacity was monitored by clonogenic soft-agar assays using bone marrow non-adherent cells as targets. The adherent stromal cells from either normal or schistosome-infected mice were able to induce the production of M, G and GM colonies from bone marrow cells (Fig. 5). Accordingly, the supernatants of the stromal cells produced similar results (not shown), indicating that the growth factors that were essential for proliferation and differentiation of myeloid progenitors were produced by omentum stroma, as well as the accessory molecules that are known to be required for myeloid progenitor cell proliferation in cultures over haemopoietic stromas (Gupta et al. 1998; Carvalho et al. 2000).

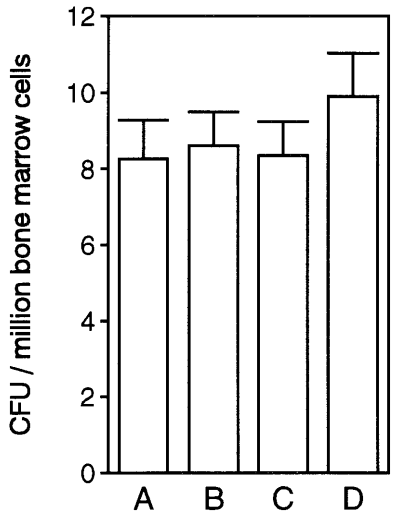

Fig. 5 CSF activity of omentum-derived stromal cells. Numbers of total CFUs (M- G- and GM-CFU) of bone marrow cells in soft-agar cultures above stromal cells established from the normal omentum (*A*), and from omenta of mice with acute (*B*) and chronic (*C*) schistosomiasis. Control experiments with GM-CSF, in the absence of stromal cells, are also shown (*D*). Results represent mean values and standard errors of three independent experiments

In order to assess whether omentum-derived stroma could sustain a long-term myelopoiesis in Dexter-type cultures, we prepared stromas composed of omentum adherent cells, and inoculated them with fresh bone marrow non-adherent cells. Under Dexter conditions, these cultures maintained myelopoiesis for up to 3 weeks. After this period they produced only macrophages, which proliferated extensively for apparently an unlimited period, and overgrew the other myeloid cell lineages (data not shown).

Connective tissue cells of haemopoietic environments including reticular and bone-lining cells are considered to be the major source of growth-promoting factors and mediators of haemopoiesis. In order to monitor the expression of growth factors by connective tissue cells from omentum, we prepared purified stroma cell cultures by serial trypsinisation, and we monitored the cytokine gene expression by RT-PCR (Fig. 6). This analysis detected a constitutive expression of the central haemopoietins for myeloid lineages: GM-CSF, M-CSF and IL-5. G-CSF was expressed in normal as well as in the stroma derived from mice in the chronic but not in the acute phase of infection. The two studied cytokines with a broad activity had an inverse and complementary pattern of expression. LIF was expressed only in stromas derived from normal mice, whilst SCF, which was not detected in normal mice, was expressed in schistosome-infected mice. Stroma derived from all the three experimental groups expressed the SDF-1α chemokine.

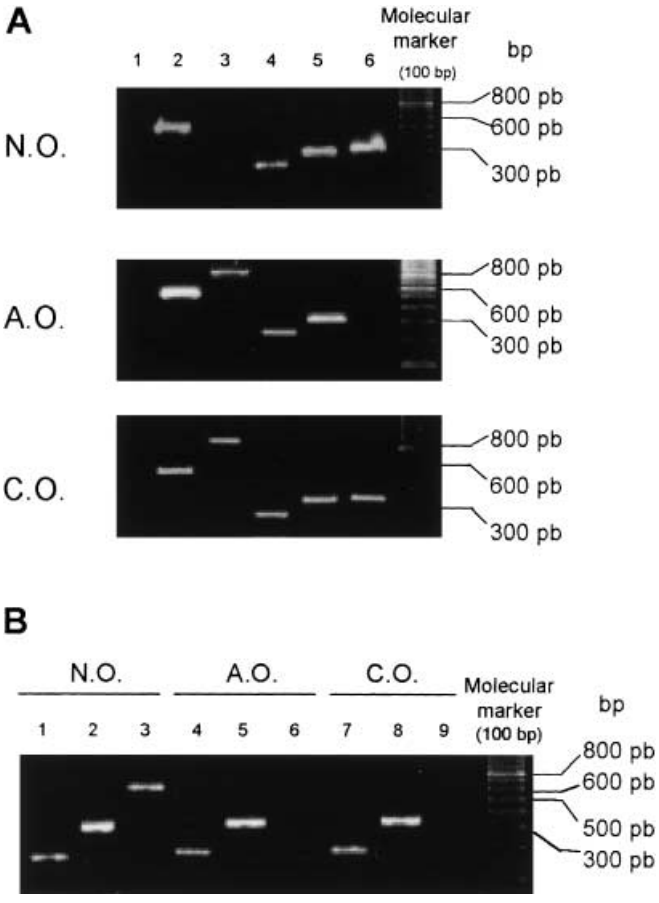

Fig. 6A, B RT-PCR analysis of cytokine expression in the omentum stroma cells of normal mice (*N.O.*), mice with acute (*A.O.*) and mice with chronic schistosomiasis (*C.O.*). **A** *Lane 1* A mock RT-PCR with RNA sample processed in the absence of reverse transcriptase, used as control for contamination with the genomic DNA; *lane 2* expression of β-actin; *lane 3* SCF; *lane 4* IL-5; *lane 5* GM-CSF; *lane 6* G-CSF. **B** Expression of M-CSF (*lanes 1, 4* and *7*), SDF-1α (*lanes 2, 5* and *8*) and LIF (*lanes 3, 6* and *9*)

Discussion

The coelom-associated lymphomyeloid tissue (CALT) is similar to the mucosa-associated lymphoid tissue (MALT) in terms of specific cellular characteristics and origin. Both tissues are formed early in embryogenesis, before the establishment of the major lymphoid cell populations that are generated by bone marrow and thymic pathways, and both harbour cell populations that are considered to be a part of primitive and natural innate resistance to adverse conditions. While MALT is essentially involved in a permanent protection of mucosae, CALT is involved in the maintenance of the homeostasis of internal cavities of the body, and it is reactive only when these cavities become the site of inflammatory reactions. In the haemopoietic system, proliferation of some of the mature cells can occur in liquid tissues such as blood, and peritoneal or pleural liquids. Conversely, progenitor cell production is associated with specific environments, where haemopoietic cells interact with stromal cells, the

locally produced or exogenous growth factors, and the extracellular matrix (Friedrich et al. 1996).

Omentum has been shown to produce several myeloid lineages, and it is able to respond to specific local demands (Cranshaw and Leak 1990; Hirai et al. 1992; Takemori et al. 1994). Milky spots are considered to be the source of peritoneal macrophages under normal conditions, and in regeneration after macrophage depletion (Biewenga et al. 1995; Zhu et al. 1997). Macrophage differentiation in the omentum tissue is apparently supported by local production of M-CSF (Ratajczak et al. 1987; Zhu et al. 1997). Previous studies have shown an increase in number and size of milky spots, as well as of free peritoneal and omentum cells, under several inflammatory stimuli (Beelen et al 1980a; Vugt et al. 1996). Accordingly, we have shown similar results in inflammatory reactions associated with schistosomal infection (El-Cheikh and Borojevic 1990; Weinberg et al. 1992). Granulocyte-macrophage colony-forming cells were described in the omentum with chronic inflammation elicited by intraperitoneal injection of Freund's adjuvant (Muller and Yoshida 1996), indicating the presence of bipotent precursors cells under such conditions. However, the presence of a specific haemopoietic or myelopoietic environment in the omentum, as well as the broad differentiation potential of progenitor cells that may reside in it, were not fully characterised, and this was the object of the present study. Here we show that omentum harbours pluripotent myelopoietic progenitors able to generate all the myeloid lineages including megakaryocytes, mast cells, and erythrocytes, when stimulated in vitro. These progenitors were relatively rare in normal omentum, and greatly increased in inflammatory reactions to schistosomal infection processes.

Mesenterium and omentum anlage are derived from the para-aortic splanchnopleura, which is a very early site of intraembryonic myelo- and lymphopoiesis (Godin et al. 1995; Medvinsky and Dzierzak 1996). Recently, elegant studies have shown that although omentum (like liver and spleen) is haemopoietic in the embryo, it is not a source of long-term totipotent haemopoietic stem cells (Godin et al. 1999). Hence, it depends upon the exogenous input of stem cells and/or progenitor cells. Accordingly, we have shown that omentum-derived stroma was able to sustain a short-term myelopoiesis in the Dexter-type culture, but not the long-term one. It should be noted that this stroma elicits and sustains an intense and long-term proliferation of macrophages in vitro, which may have overgrown the earlier progenitors. Since this reflects the natural function of the omentum in vivo, we consider that it corresponds to the natural pattern of myelopoiesis in omentum.

Molecular study of gene expression in the omentum stroma has shown a constitutive expression of SDF-1α, a chemokine that is known to elicit homing of haemopoietic progenitors (Aiuti et al. 1997; Jo et al. 2000). This is in agreement with recent reports showing that SDF-1α is constitutively expressed in the mesothelial cells from peritoneal, pleural and pericardial serosae in adult mice (Foussat et al. 2001), which have the same mesodermal origin. Early haemopoietic progenitors are normally found in low numbers in circulation, and may be attracted to the omentum stroma, maintaining a basal production of myeloid cells. In general, inflammatory reactions cause an increased production of cytokines such as G-CSF and GM-CSF, which elicit mobilisation of haemopoietic stem cells into the peripheral circulation (Young and Cheers 1986; Roberts and Metcalf 1994). The increased number of myeloid progenitors in the inflamed omentum may reflect the increased availability and input of these cells. SDF-1α affects the function of adhesion receptors including β_1 and β_2 integrins, allowing stem and progenitor cells to adhere to the endothelium expressing SDF-1α (Weber et al. 1996; Naiyer et al. 1999). Moreover, SDF-1α induces vascular endothelial growth factor (VEGF) secretion (Kijowski et al. 2001). We have previously described increased vascularisation of mesenteric milky spots in schistosomiasis (Weinberg et al. 1992), and we have now observed the same in omentum (unpublished).

We have also found that normal omentum stroma produced LIF, a growth factor associated with proliferation of early progenitors, which can sustain their slow steady-state renewal and maintenance of their stemness under normal conditions (Tanosaki et al. 1999). In the presence of M-CSF that is constitutively expressed in omentum stroma, LIF can induce early progenitor cells to myeloid differentiation sustaining the steady-state production of peritoneal macrophages (Keller et al. 1996). LIF is also an anti-inflammatory cytokine (Banner et al. 1998) and it is noteworthy that its expression is downregulated in omentum stroma under abdominal inflammatory reactions, in which it is substituted by SCF. This factor is a potent chemoattractant when combined with SDF-1α (Kim and Broxmeyer 1998) and a potent growth factor for several myeloid cell lineages when acting in collaboration with the central haemopoietins (Migliaccio et al. 1991; Tsuji et al. 1991). This shift may release the early progenitors located in omentum from their low-renewal state, and induce their intense expansion leading to the observed increase in committed stem cells for the major myeloid lineages involved in inflammation. Simultaneously, the increased availability of circulating early progenitors can supply their permanent renewal. The late expansion is mediated by constitutive expression of the central haemopoietins IL-5, GM-CSF, M-CSF and G-CSF. The major control of the increased inflammatory cell production in omentum would be thus the result of the input and expansion of early progenitors, rather than the mobilisation of late progenitors or mature inflammatory cells from the systemic pool. The delay in induction of the progenitor increase, and the lack of its correlation with the systemic increase in, e.g., eosinophils, favours this hypothesis.

In conclusion, our study has shown that the normal omentum harbours pluripotent myeloid precursors, able to originate all the myeloid cell lineages, as well as the appropriate stromal environment able to sustain it. In-

flammatory reactions, and in particular the chronic inflammation, increase the number of progenitors and of locally produced myeloid cells, through a complex modulation of the myelopoietic microenvironment and production of chemotactic and growth factors. These modifications generate conditions appropriate for increased local production of inflammatory cells in the abdominal cavity.

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