Haematology and Blood Transfusion Vol. 29
Modern Trends in Human Leukemia VI
Edited by Neth, Gallo, Greaves, Janka

Proliferation In Vivo and In Vitro of Haemopoietic Progenitor Cells Induced by AF-1, a New *ras*-Containing Retrovirus

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A. Introduction

Many studies have been performed on the effects of murine retrovirus infection upon the haematopoietic system. In some instances following a variable period after in vivo infection transplantable tumours or continuous cell lines have been developed [1, 2]. The cell lines appear to proliferate independently of haemopoietic growth factors, although the possibility remains that the transformed cells are able to produce their own growth factors. In most cases of transformation of haemopoietic cells and production of continuous cell lines by retroviruses the target cell for viral transformation remains unknown.

Although several in vitro infection systems have been developed [3, 4], these contain both haemopoietic and non-haemopoietic cells and thus make the interpretation of target cell type impossible. The present experiments were performed to answer some of these questions using a newly isolated murine retrovirus AF-1. AF-1 is a replication defective retrovirus derived from passage of cloned F-MuLV through newborn BALB/c mice [5]. It contains a *ras* oncogene related to that of Ha-*ras* [5]. Infected animals develop a rapid splenomegaly, associated with increased levels of splenic haemopoietic progenitor cells and a histiocytosis [5]. Cell lines derived from AF-1-infected spleen cells are capable of producing G-CSF and GM-CSF but not multi-CSF (IL-3) (W. Ostertag, G. W. Johnson, unpublished observations).

B. Results

I. Growth of "Factor-Independent Colonies" from AF-1-Infected Spleen and Bone Marrow

DBA mice were infected with AF-1 and 14 days later spleen and bone marrow cells were cultured in semisolid agar medium [6]. Cells in half of the cultures were stimulated by the addition of pokeweed mitogen-stimulated spleen-cell-conditioned medium (SCM) [7]. When scored at 7 days, the frequency and distribution of colony types in stimulated infected bone marrow cultures did not differ significantly from control non-infected cultures. Infected spleen-cell cultures when stimulated contained an approximately tenfold higher frequency of colonies than control cultures. Cultures of infected spleen or bone marrow cells without stimulus displayed cell dose-dependent "factor-independent" colony formation. Thus with spleen cells cultured at 1, 2 and 4×10^5 cells, 0, 7 ± 1 and 56 ± 2 colonies were obtained (control cultures of 1×10^5 cells stimulated with SCM contained 121 ± 19 colonies) including macrophage, neutrophil, erythroid and multipotential colonies (see Table 1). Sequential "factor-independent" colonies were removed from

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Table 1. Colony types producing cell lines

Colony source		Number of colonies transferred	Number of colonies with proliferating cells
Bone marrow	Macrophage	24	3
	Neutrophil-macrophage	20	9
	Macrophage-blast-mast	1	0
	Erythroid-macrophage	2	0
	Macrophage-erythroid-blast	2	0
Spleen	Macrophage	29	14
	Neutrophil-macrophage	12	4
	Neutrophil	1	0
	Blast	1	0
	Neurophil-macrophage-erythroid	2	0
	Neutrophil-macrophage-blast-mast	2	1

Colonies isolated from 7-day cultures of 1×10^5 cells obtained from CBA or DBA mice infected with AF-1 14 days previously. Proliferation of colony cells determined at 14 days and in all cases consisted of elongated adherent cells

spleen and bone marrow cultures and half of each colony was recultured in 200 µl medium to determine the ability for continued proliferation of colony cells. The remaining cells from each colony were smeared and stained to determine colony morphology. The results of one experiment are shown in Table 1. Continued proliferation of colony cells was observed from macrophage and neutrophil-macrophage colonies and occasional multipotential colonies (Table 1). After 14 days, medium from wells containing proliferating colony cells was assayed and found to contain no detectable GM-CSF, G-CSF or multi-CSF. Cells continued to proliferate until the experiment was terminated at 8 weeks after initial colony-cell transfer and at no stage were any haemopoietic growth factors detected in supernatants from the proliferating cells. Cells from all factor-independent colonies induced foci in cocultures with indicator fibroblasts, indicating the presence of virus.

II. Effect of AF-1 on Single Haemopoietic Progenitor Cells

To determine whether the effect of AF-1 on haemopoietic progenitor cells was direct or indirect single cells obtained from fractions highly enriched for colony-forming cells and purified from CBA fetal liver as described previously [8] were cultured in microtitre wells in the presence of virus. The results of these experiments are shown in Table 2. Single cells were transferred to wells containing medium alone or SCM, SCM plus AF-1 and AF-1 alone. When scored 7 days later, as expected no cells were present in medium-alone controls and over 20% of wells with SCM contained proliferating cells. The addition of the supernatant containing dialyzed AF-1 to SCM cultures markedly inhibited proliferation. AF-1 supernatant alone was able to induce limited proliferation from single cells but after 14 days all proliferation had ceased in these wells (Table 2).

C. Discussion

Mice infected with AF-1 show marked splenomegaly and elevation of splenic haematopoietic progenitor cells. The present experiments suggest that this may in part be a direct proliferative effect of the virus complex upon progenitor cells. However, a more significant effect, at least during the

Table 2. Effect of AF-1 on single haemopoietic progenitor cells

Addition to well	Medium	SCM	SCM + AF-1	AF-1
Proliferating clones per number of single cells transferred	0/120	27/119	9/96	6/95
Cell number per clone at 7 days (range)	0	9–560	2–16	4–74

Single cells from progenitor-cell-enriched fraction (CFC fraction, see [8]) micromanipulated into 200 µl medium alone or containing SCM and/or AF-1

early stages of infection, may be mediated by the activation of accessory-cell-derived haemopoietic growth factor synthesis. This would be in agreement with the observation of cell-number-dependent colony formation in spleen or bone marrow cultures from which SCM was omitted. The non-linearity of colony formation coupled with the fact that less than 50% of the colonies contained cells capable of further proliferation suggests that non-proliferating cells are producing the stimuli required for proliferation.

The continued proliferation of colony cells from macrophage and neutrophil-macrophage colonies suggests that granulocyte-macrophage progenitor cells (GM-CFC) may be the primary target cell for AF-1-induced transformation. The presence of virus-inducing fibroblast transformation in all "factor-independent" colonies probably indicates that all cells can be infected with AF-1. Furthermore, the continued proliferation of cells from one multipotential colony (containing neutrophils, macrophages, blast cells and mast cells) together with the association of proliferation with macrophage differentiation may suggest that infection of all cell types can occur, but that commitment to macrophage differentiation may be important for transformation and continued proliferation of cells.

Although accessory cells probably play a role in AF-1-induced haemopoietic proliferation the experiments with single cells suggest that the AF-1 viral complex itself may be able to induce limited proliferation directly. Further experiments with more purified viral preparations are required to clarify this point although in the experiments reported here the viral supernatants were passaged through membranes to exclude molecules of less than 300 000 daltons and were shown to be negative for haemopoietic growth factors by bioassay. This latter treatment is important as most fibroblasts used for the maintenance of cloned virus preparations produce growth factors [9] (G.R. Johnson and W. Ostertag, unpublished observations).

In summary, the experiments reported here suggest that the AF-1 virus complex is able to induce haemopoietic-cell proliferation by both direct and indirect means and that factor-independent cell proliferation may be related to macrophage differentiation. Further experiments are required to determine the role of the AF-1 ras gene on these processes.

Acknowledgments. Part of this work was supported by the Carden Fellowship Fund of the Anti-Cancer Council of Victoria, the J.D. and L. Harris Cancer Fund, the National Health and Medical Research Council, Canberra, and the National Institutes of Health, Bethesda, Grant No. CA-25972. Another part (W.O.) was funded by the Deutsche Forschungsgemeinschaft and by the UICC. The Heinrich-Pette-Institut is supported by Freie und Hansestadt Hamburg and Bundesministerum für Jugend, Familie und Gesundheit. – The excellent technical assistance of Misses E. Viney and U. Bergholz is gratefully acknowledged.

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