Pathogenicity of Trypanosomes

Proceedings of a workshop held at Nairobi, Kenya, 20-23 November 1978

Editors: George Losos and Amy Chouinard
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¹IDRC project coordinator, Veterinary Research Department, Muguga, Kenya.
²Editor, Communications Division, IDRC, Ottawa, Canada.
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Losos, G.
Chouinard, A.
Kenya Agricultural Research Institute, Veterinary Research Dept., Muguga KE
IDRC, Ottawa CA
International Laboratory for Research on Animal Diseases, Nairobi KE
CIDA, Ottawa CA


/IDRC publication/. Compilation of workshop papers on /trypanosomiasis/ particularly in /Africa south of Sahara/ - discusses the /metabolism/ of the trypanosome /parasite/s, mechanisms of /disease transmission/, effects on /blood/ and /serum/ /protein/ levels in /cattle/, /immunology/cal aspects, /disease resistance/.

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Effect of bovine trypanosomiasis on hematopoiesis

G.P. Kaaya, G.J. Losos, M.G. Maxie, and V.E.O. Valli

Veterinary Research Department, Kenya Agricultural Research Institute, Muguga, Kenya, and Department of Pathology, Ontario Veterinary College, Guelph, Canada

Abstract. Bovine granulocyte/macrophage colonies ranging from 20 to 1000 cells were grown in methyl cellulose cultures. The percentage of adherent cells in bovine marrow was found to vary from 20 to 66%. Bovine erythroid colonies were cultured in plasma clots. Sera collected from cattle infected with Trypanosoma vivax or T. congolense inhibited the growth of granulocyte/macrophage colonies but not the erythroid colonies. Moreover, marrow collected from calves infected with T. congolense (TREU 112) showed a decreased efficiency of granulocyte macrophage colony formation in vitro. The future prospects of in vitro bone marrow culture as a tool for studying animal hematopoietic diseases are discussed.

Bradley and Metcalf (1966) in Australia and Pluznik and Sachs (1966) in Israel were the first to report the in vitro culture of mouse CFU-C colonies in agar. The progenitors of these colonies were designated "colony forming units in culture or CFU-C." Thereafter, the CFU-C colonies were successfully grown from human (Senn, McCulloch, and Till 1967), rat (Bradley and Siemienowicz 1968), bovine (Walker, Valli, and Lumsden 1974), and dog (Kovacs, Brunch, and Fliedner 1976) marrow cells. The growth of CFU-C colonies requires stimulation by colony stimulating activity (CSA), a heterogeneous group of glycoproteins. Price, McCulloch, and Till (1975) purified CSA from medium that had been conditioned by normal human peripheral leukocytes and observed that the CSA contained three molecular species of approximately 100,000, 35,000, and 15,000 daltons. CSA has been produced from a variety of sources, e.g., from human peripheral leukocyte conditioned medium (Iscove et al. 1976; Price, McCulloch, and Till 1973), mouse kidney cells (Bradley and Summer 1968), human urine (Metcalf 1971; Stanley et al. 1977), animal postendotoxin injection sera (Bierman and Hood 1972; Chervenick 1972), and postirradiation sera (Beran 1975; Laissue et al. 1975). The principal source of CSA is the monocyte/macrophage type of cell (Chervenick and LoBuglio 1972; Golde, Cline, and Finley 1972), and endotoxin acts directly on this cell to stimulate CSA production (Goldman 1975). Price, McCulloch, and Till (1975) demonstrated that CSA from human peripheral blood leukocytes is a product of cell membranes.

In our experiments, bovine CFU-C cultures were prepared in methyl cellulose. Large colonies ranging in size from 20 to 1000 cells were produced within 6-7 days in culture. Bovine serum after endotoxin injection was used as the source of CSA. Our results confirmed that injection of animals with endotoxin increases serum levels of CSA significantly.

Messner, Till, and McCulloch (1973) observed that human marrow cell suspensions contain two different populations of cells, one population capable of adhering (adherent cells) and one incapable of adhering to glass and plastic (nonadherent cells). They observed that the adherent cells produce CSA and that their proportion in human marrow cells varies from 20 to 50%. In bovine marrow cells, we have found that the proportion of adherent cells varies from 20 to 60% and that there may be no marked difference between humans and cattle in the percentage of these cells. Moreover, no difference was observed between control and T. vivax-infected cattle in the percentage of adherent cells.
Erythroid (CFU-E) Colony Culture

Stephenson et al. (1971) were the first to report the in vitro culture of mouse erythroid colonies. They designated the cells originating these colonies as “erythroid colony forming units or CFU-E.” They used a plasma clot technique, which was slightly modified later by McLeod, Shreeve, and Axelrad (1974) using microwells instead of petri dishes. The technique has since been successfully applied to human (Tepperman, Curtis, and McCulloch 1974), dog (Brown and Adamson 1977), and bovine (Kaaya, Valli, and Maxie 1978) marrow cells. CFU-E colonies have also been successfully grown in methyl cellulose using mouse (Hara and Ogawa 1977; Iscove and Sieber 1975) and human (Iscove, Sieber, and Winterhalter 1974; Ogawa et al. 1977) marrow cells. We have successfully grown bovine CFU-E colonies in plasma clots, using both the original method and the modified technique. However, we were unable to grow colonies in methyl cellulose cultures. Growth of bovine CFU-E in methyl cellulose appears to require culture conditions different from those described for mouse and human CFU-E.

Clinical Applications

The development of CFU-C culture technique by Bradley and Metcalf (1966) and Pluznik and Sachs (1966) and of CFU-E by Stephenson et al. (1971) opened a new era in clinical hematology. A few hematological diseases have already been studied using in vitro bone marrow culture, and useful results have been obtained, e.g., marrow from humans suffering from acute myelogenous leukemia has been found to be capable of forming very few small CFU-C colonies during relapse, and the efficiency of colony formation improves during remission (Harris and Freireich 1970). Price, McCulloch, and Till (1975) observed that CSA from normal humans (peripheral leukocyte conditioned medium) contained three molecular types, i.e., 100,000, 35,000, and 15,000 daltons, but that CSA from leukemic patients in relapse contained only one molecular type, usually 35,000 daltons. In remission, leukemic patients exhibited all three types. Likewise, CSA from peripheral blood leukocytes of patients suffering from idiopathic acquired sideroblastic anemia, a disease that often develops into leukemia, contained only one molecular species (Till et al. 1975). These observations show that in vitro bone marrow culture may be a useful tool for the diagnosis of early relapses and completeness of remission and for predicting the prognosis of leukemias. The plasma clot culture has been used to study congenital hypoplastic anemia and pure red cell aplasia, and findings indicate that in congenital hypoplastic anemia, the inhibitor of erythropoiesis is located in the lymphocytes, whereas in pure red cell aplasia, the inhibitor is located in the serum (Hoffman et al. 1976). The standard agar culture for CFU-C has been used to detect inhibitors of granulopoiesis in sera of BALB/C mice (Metcalf and Russell 1976) and in dialysates of media conditioned by normal and leukemic mouse cells (Metcalf 1971) and to demonstrate the inhibitory effect of anti-mouse brain serum in vitro or mouse CFU-C colony formation in vitro (Meyer-Hamme and Bluestein 1978).

To date, in vitro bone marrow culture has not been used widely for studying animal diseases, but in future, it will likely be used for studying diseases associated with bone marrow depression anemias and myeloproliferative disorders. In our experiments with in vitro bone marrow culture, we found that sera from T. vivax- and T. congolense-infected cattle inhibited granulopoiesis but not erythropoiesis (Table 1). The nature and source of the inhibitor are not yet known, but further work is being done to characterize it. Marrow collected from calves infected with T. congolense (TREU 112) shows a decreased efficiency of CFU-C colony formation (Table 2). Similar results were reported by Valli, Mills, and Lawson (1977). It is concluded that the stem-cell pool in the calves was reduced — a finding that suggests the inhibitor of leukopoiesis kills stem cells. We strongly believe that the leukopenia reported in cattle infected with T. congolense...
Table 2. A comparison of CFU-C colonies from *T. congolense*-infected and control calf marrow samples (marrow was collected 3 weeks post infection).

<table>
<thead>
<tr>
<th>Cell concentrations</th>
<th>Control calf (no. 3)</th>
<th>Infected calf (no. 4)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.5</td>
<td>0.8</td>
</tr>
<tr>
<td>Colonies –</td>
<td></td>
<td></td>
</tr>
<tr>
<td>dish 1</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>dish 2</td>
<td>4</td>
<td>10</td>
</tr>
<tr>
<td>dish 3</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>dish 4</td>
<td>2</td>
<td>7</td>
</tr>
<tr>
<td>Mean per dish</td>
<td>2.25</td>
<td>7.00</td>
</tr>
</tbody>
</table>

*Mean per dish* 0.25(NS)*a* 1.25(S)*a* 1.75(S)*a* 4.50(S)*a* 5.00(S)*a* 37.25(S)*a* 53.50(S)*a* 81.25(S)*a*

*aS = significant (P < 0.05); NS = not significant.

(Losos et al. 1973; Naylor 1971) and *T. vivax* (Maxie, Losos, and Tabel 1976; Vohradsky 1971) results from loss of stem cells caused by circulating toxic factors. The fact that no inhibitor of erythropoiesis was detected in sera collected from our trypanosome-infected cattle does not confirm the absence of an erythropoietic inhibitor because the CFU-E is a much more mature progenitor than is CFU-C (Gregory, McCulloch, and Till 1973), and, therefore, the CFU-E might have differentiated beyond the inhibitable stage. It is also possible that the inhibitor, if present, is located in lymphocytes as is the case in congenital hypoplastic anemia (Hoffman et al. 1976).