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Surface Antigens of Pluripotent and Committed Haemopoietic Stem Cells

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A. Introduction

Blood cells are formed from stem cells which occur in low numbers in the bone marrow and spleen. A certain fraction of the stem cells is capable of giving rise to all types of blood cells. These are defined as the "pluripotent stem cells". A more mature cell type which is still capable of extensive proliferation but which is restricted in its maturation to a single blood cell line is defined as the "committed stem cell".

In mice the two types of stem cells can be demonstrated in different assay system. The pluripotent cells are measured in the spleen colony assay or CFU-S assay (Till and McCulloch 1961). A number of in vitro culture systems are available to detect committed stem cells. These culture techniques depend on the addition of growth regulators for a particular blood cell line. In the experiments that are reported here in vitro colony forming cells (CFU-C) which grow upon exposure to CSF were studied (Bradley and Metcalf, 1965). These CFU-C are considered to represent stem cells which are committed to granulocyte/macrophage differentiation.

The differentiation from pluripotent into committed stem cells is not accompanied by a morphologic change. Cell separation studies have shown that CFU-S and CFU-C are very similar in their overall morphologic characteristics and that they cannot be separated by their physical properties.

In this paper some cell surface antigens are studied. The results show that the differentiation of pluripotent stem cells to committed cells is accompanied by changes in cell surface antigen density.

B. Materials and Methods

Details about the colony assays for haemopoietic stem cells and handling of the bone marrow cells in suspension are described elsewhere (Till and McCulloch 1961; Bradley and Metcalf 1966; Van den Engh 1974). The cytotoxicity of an antiserum was determined by incubating cells for 30 min with the serum at 0°C followed by incubation with rabbit complement for 30 min at 37°C. Cell sorting was done on a FACS II (Becton and Dickinson). The cells were labeled by incubating them with a DNP-labeled antibody followed by incubation with an FITC-labeled antibody against DNP. Except for the rabbit anti-mouse brain sera (Golub 1972), the sera were raised in congenic mouse strains (Trask and Van den Engh 1980).

C. Results

The expression of cell surface antigens on haemopoietic stem cells was investigated in two ways. In one method mouse bone marrow cells were treated with antisera and complement to see whether CFU-S and CFU-C would be affected by this treatment. Experiments of this type led to the conclusion that the CFU-S shared an antigen with mouse brain tissue. This antigen was not found on CFU-C (Golub 1972; Van den Engh and Golub 1974). The same procedure showed both CFU-S and CFU-C to be negative for the Thy-1 antigen. Thus, a potential differentiation antigen which discriminated between pluripotent and committed stem cells was described. Using the same methods, other antigenic differences between CFU-S and CFU-C were observed. K and D region antigens of the H-2 complex are abundently expressed on the CFU-S and are present at much lower densities on CFU-C.

I region antigens were found to be absent on both cell types (Russell and Van den Engh 1979).

Some uncertainty about the proper interpretation of these results remained. The inhibition of spleen colony formation of pluripotent stem cells by antibody treatment also occurs in the absence of complement. Therefore, the failure to abolish in vitro colonies did not unequivocally demonstrate an antigenic difference.

In a second series of experiments the surface antigens of the CFU-S and CFU-C were studied by measuring the binding of fluorescent antibodies in a cell sorter. The results obtained with anti-H2 sera and anti-Thy-1 sera confirmed the conclusions which were drawn on the basis of the cytotoxic properties of the antisera. Figures 1 and 2 show the fluorescence distribution of mouse bone marrow cells after treatment with DNP-labeled α-H2 or α-Thy-1 followed by incubation with an FITC-labeled α-DNP antibody. The figures also sow the relative distribution of CFU-S that is observed after the cells are sorted into fractions of different fluorescence intensity. As in cytotoxicity studies, Thy-1 is not present in appreciable amounts on the CFU-S surface and H-2 antigens are present at high densities.

Similar experiments with rabbit anti-mouse brain serum fal to show a preferential binding of these sera to CFU-S. Figure 3 shows the fluorescence intensity profiles of bone marrow cells treated with an α-brain-DNP α-DNP-FITC sandwich. Most bone marrow cells bind some amount of the antisera. Only a small proportion of cells can be considered to be strongly positive. When the cells are sorted according to fluorescence intensity, the CFU-S are found among the weakly positive cells. Therefore, CFU-S do bind some of the antibody, but this binding is by no means specific. The abolishment of the CFU-S in vivo must therefore be due to a particular property of the in vivo assay rather than specificity of the antiserum.

D. Discussion

The demonstration of surface antigens on pluripotent haemopoietic stem cells in the mouse (CFU-S) was found to be dependent on the method used. When the cytotoxic properties of the antisera were used to demonstrate binding of antibody, heterologous anti-mouse brain serum seemed to react specifically with CFU-S. However, in experiments in which the affinity of fluorescent-labeled anti-mouse brain serum was measured, no preferential binding to CFU-S was observed. Since the cytotoxic effect of anti-mouse brain serum does not depend on complement treatment, the most likely explanation is that CFU-S suppression is due to mechanisms which are particular for the CFU-S assay rather than due to the presence of a differentiation antigen specific for pluripotent stem cells (Trask and Van den Engh 1980).

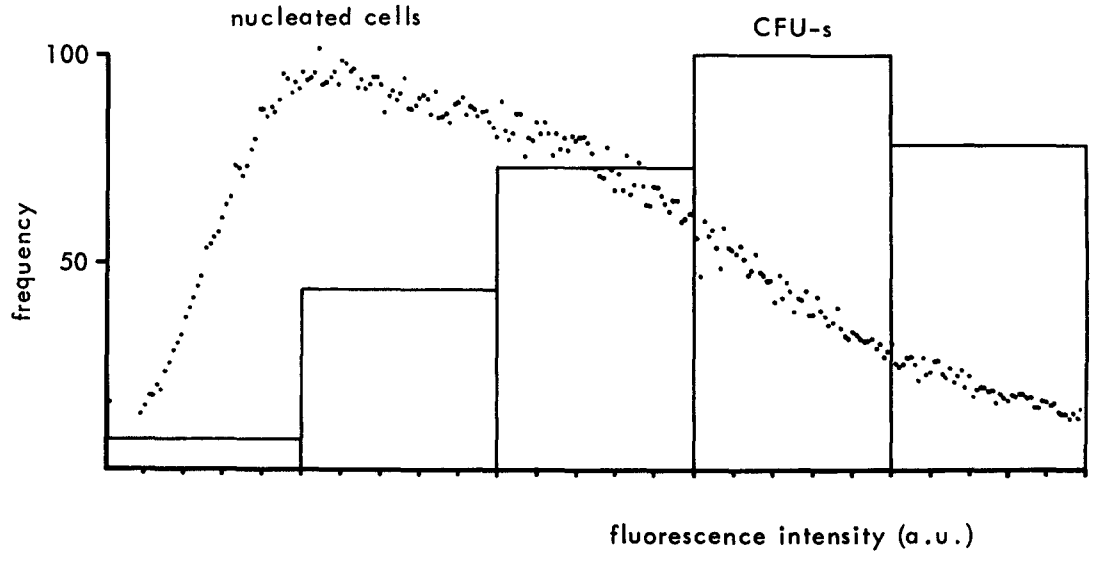

Fig. 1. Fluorescence distribution of CFU-S compared to that of viable bone marrow cells after treatment with anti-H-2-DNP-γG followed by anti-DNP-FITC. The *dots* give the fluorescent distribution of viable bone marrow cells. The *histogram* gives the numbers of CFU-S found in fractions of increasing fluorescence intensity (corrected to 100% peak value). *a.u.*, arbitrary units

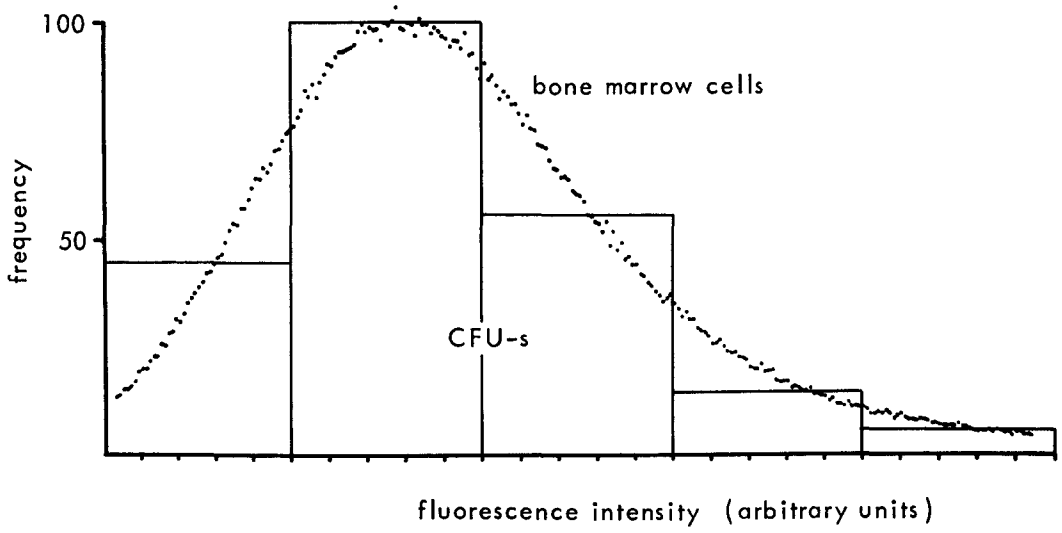

Fig. 2. Fluorescence distribution of CFU-S compared to that of viable bone marrow cells after treatment with anti-Thy-1.2-DNP-γG and 1/6 anti-DNP-FITC. The *dots* give the fluorescence of viable bone marrow cells. The *histogram* gives the numbers of CFU-S found in fractions of increasing fluirescence intensity (corrected to 100% peak value)

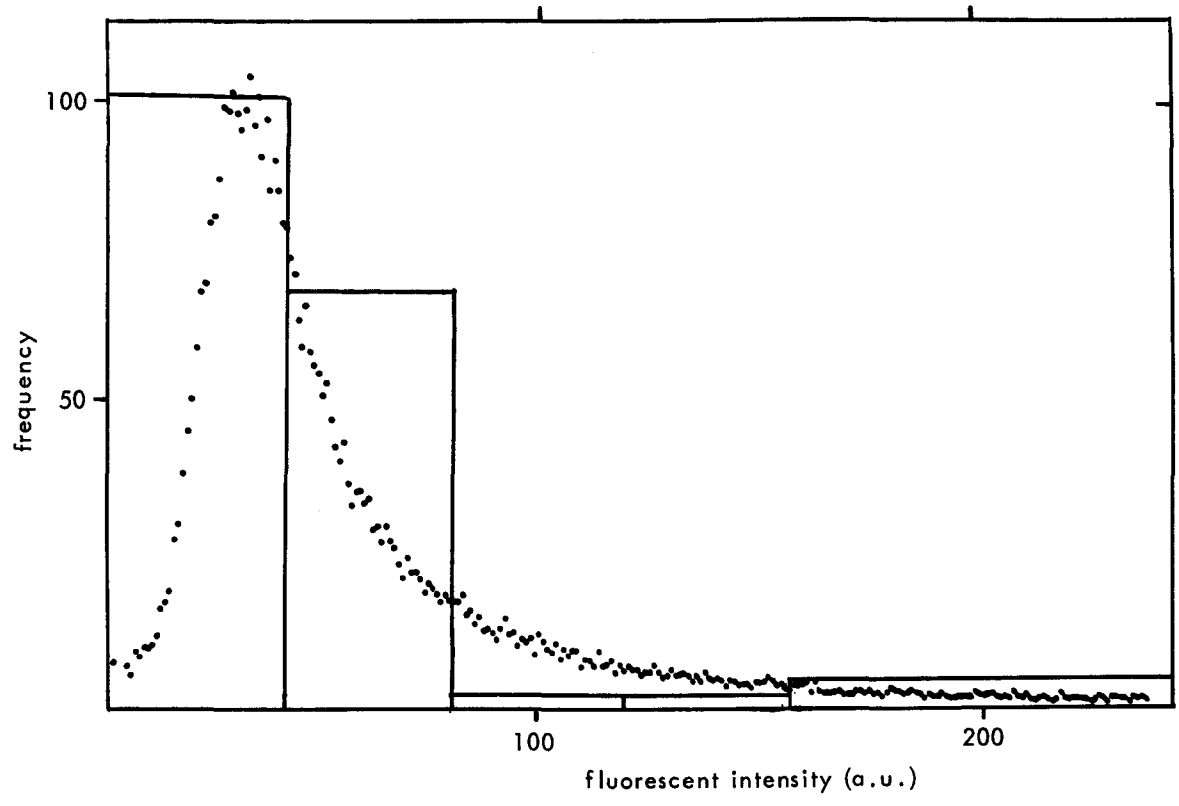

Fig. 3. Fluorescence distribution of CFU-S compared to that of viable bone marrow cells after treatment with RAMBR-DNP-γG followed by anti-DNP-FITC. The *dots* give the fluorescence distribution of viable bone marrow cells. The *histogram gives the numbers of CFU-S found in fractions of increasing fluorescence intensity (corrected to 100% peak value). a. u.*, arbitrary units

However, the results obtained with cytotoxicity test using congenic mouse sera were confirmed by flow cytometry in a cell sorter. These methods show that in the mouse Thy-1 is not present on CFU-S. CFU-S has a high density of H-2 KD antigens and are comparable in this property to spleen lymphocytes. H-2 I antigens are not yet expressed on these early cells. This pattern of antigen expression is probably species specific. Thy-1 has been reported to be expressed by the CFU-S in rat bone marrow (Goldschneider et al. 1978). This observation has been confirmed in our laboratory. In the human I region antigens

have been reported to be present on CFU-C. The abundance of H-2 KD antigens may be of particular interest. Y. L. Weissman (personal communication) showed that this is also characteristic for thymic precursor cells and thus may be a common feature of the earliest blood cells of the mouse.

Acknowledgments

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