HUMAN MYELOID DIFFERENTIATION ANTIGENS

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ABSTRACT

This thesis is concerned with the identification and characterization of antigens defined by monoclonal antibodies (MoAb's) that can be used in the investigation of human myeloid haemopoiesis and differentiation.

A model system for the study of human monocytic differentiation was investigated. It was found that cells of the human myelomonocytic cell line RC-2A would differentiate towards macrophages when treated with a source of lymphocyte derived factors obtained from mitogen stimulated human peripheral blood mononuclear cells.

A panel of MoAb's was used to investigate the surface marker changes involved in this process, and in addition, new MoAb's were produced which were myeloid specific. A colorimetric assay for screening MoAb's to cell surface antigens was modified to enhance its sensitivity, in an attempt to maximise the chances of identifying MoAb's binding to antigens of low copy number.

Cells of the human myelomonocytic line RC-2A were induced to differentiate towards macrophages by culturing for up to twelve days in the presence of supernatant from phytohaemagglutinin stimulated human peripheral blood mononuclear cells (PHA-LCM). The process of differentiation was monitored by changes in expression of two macrophage related enzymes (a-Naphthol Butyrate Esterase and Acid Phosphatase), the changes in expression of the monocyte/macrophage cell surface markers detected by the monoclonal antibodies anti-Mo1 and anti-Mo2, Ia antigen detected by the monoclonal antibody FMC-14, and alteration in cell morphology.
RC-2A cells over a period of 8 days culture in the presence of PHA-LCM expressed α-Naphthol Butyrate Esterase very strongly compared to untreated cells, and de novo Acid Phosphatase activity was found within lysosomal granules. Such a pattern of enzymic expression is typical of macrophages. Investigation of cell surface markers detected by MoAb's showed changes consistent with differentiation towards macrophages, with the binding of α-Mo1 and α-Mo2 increasing. Induced RC-2A cells were able to stimulate in one-way Mixed Leukocyte Culture more effectively than control cells. Maturation induced by PHA-LCM was accompanied by a marked decrease in the proliferative potential of the cell population, and a reduced ability to form colonies in semi-solid medium. After 9 days in culture with PHA-LCM, these RC-2A cells had only 3% of the clonogenic potential of control untreated cells, suggesting that differentiation was accompanied by an irreversible loss of proliferative ability.

Investigations into the effects of the recombinant human biological factors tumour necrosis factor (TNF), Y-Interferon, Granulocyte-Macrophage colony stimulating factor (GM-CSF) and Granulocyte colony stimulating factor (G-CSF) revealed that none of these alone could induce the full complement of differentiation effects mediated by PHA-LCM, suggesting full differentiation is likely to be a multifactorial process. For example, of the four factors investigated, only Y-Interferon and G-CSF were able to reduce the clonogenic potential of RC-2A cells, and only Y-Interferon was able to increase the expression of α-Naphthol Butyrate Esterase. The alteration of antigen expression on RC-2A cells mediated by Y-Interferon and TNF was quite different to the pattern of expression induced by PHA-LCM.
A polyclonal antiserum against RC-2A cells raised in mice and absorbed with the autologous B lymphocyte cell line Cess B, was shown to increase the proliferation of RC-2A cells at appropriate dilutions, demonstrated by a 3 fold increase in \(^{3}H\)-thymidine uptake. The antiserum was also able to stimulate the formation of exclusively macrophage colonies in semi-solid agar culture of human bone marrow mononuclear cells. These properties were not shared by normal mouse serum, suggesting the feasibility of raising monoclonal antibodies with functional effects on haemopoietic cells.

In an effort to isolate MoAb's which exerted functional effects on RC-2A cells, in addition to those recognising human myeloid differentiation antigens, a screening assay was required which would identify antibodies binding antigens of low copy number. A previously described assay for detecting MoAb's binding to cell surface antigens, called the Rose Bengal Assay (RBA), was modified to give increased sensitivity. This assay involved coating the wells of 96-well microtitre trays with an anti-murine immunoglobulin reagent, allowing any immunoglobulin in test hybridoma supernatants to bind. Then target cells were added, which would be bound to wells that contained specific antibody from the hybridoma supernatant. Bound cells were stained with Rose Bengal dye, and optical density at the relevant wavelength correlated with presence of antibody specific to the target cells. During the course of these investigations, a number of antibodies reproducibly demonstrated binding to target cells in the RBA, but gave negative or very weak binding in indirect immunofluorescence assay, and would probably have been overlooked had such an assay been used for the initial screening process.
Hybridomas were produced by fusion of spleen cells from mice immunised with RC-2A cells with cells of the murine myeloma line x63Ag8.653, and selection in HAT medium. Hybrids were screened using the RBA, and those which produced antibody binding to RC-2A, but not to the autologous B lymphocyte cell line Cess B, were chosen for cloning by limit dilution and further investigation. Eventually, five differing MoAb's were selected to be characterised more fully. These MoAb's were screened for functional effects on RC-2A cells and haemopoietic cells, but were found to have no activity in the systems studied. The binding pattern of these five MoAb's to human haemopoietic cell lines, normal blood cells and tissue sections was determined, and their specificity for human haemopoietic progenitors was studied using negative selection by complement mediated cell lysis. The molecular weights of antigens detected by the MoAb's were determined for four of the five antibodies. Comparisons between these antibodies and those described in the literature suggest that the specificities of two of the MoAb's obtained may be different to those of antibodies previously published.
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