THE EFFECT OF CELL SYNCHRONIZATION UPON THE DETECTION OF T AND B LYMPHOID CELL RECEPTORS ON TWO CONTINUOUS LYMPHOID CELL LINES

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SUMMARY

In the present study, the effect of the cell synchronization on the detection of T and B cell surface markers of two continuous lines of lymphoid cells (FL-74 and CT45-S) was examined. Suspension cultures were synchronized by deprivation of isoleucine and surface markers were quantitated by T rosette formation with guinea pig erythrocytes (E) and B rosette formation with an erythrocyte-antibody-complement (EAC) complex. After 24 hr, cells were resuspended in complete culture medium. Virtually 100% of FL-74 cells expressed the T cell marker at time 0, with a progressive decline to 80% at saturation density. A bell-shaped curve for expression of the EAC marker on CT45-S cells was seen with maximum expression in the logarithmic phase of the growth cycle. Spent culture medium was examined for the presence of free soluble receptor. Preincubation of E and EAC in appropriate old medium resulted in 42% inhibition of E rosettes and 42% inhibition of EAC rosettes with FL-74 and CT45-S cells, respectively. Thus quantitation of lymphocyte subpopulations as B, T or null cells with these cellular markers may be influenced by the age of the cell examined, phase of the cell cycle and the amount of free receptor present in the surrounding medium.

Key words: cell synchronization; lymphoblastoid cell lines; E and EAC rosettes.

INTRODUCTION

The use of cell surface markers to delineate subpopulations of lymphocytes in man and animals has received wide application in the evaluation of the role of lymphocytes in the immune response. Thymic-derived lymphocytes (T cells), concerned with cellular or delayed hypersensitivity, are identified by their ability to form nonimmune rosettes with erythrocytes of heterologous or, in some cases, homologous species (1-4). Bursal or bone marrow-derived lymphocytes (B cells), concerned with humoral immunity, possess a receptor for the third component of complement and are identified by their ability to form rosettes with an erythrocyte-antibody-complement (EAC) complex (5-8). There is general agreement that these cellular populations are non-overlapping.

In in vitro studies, the cell cycle has been found to influence the production of many cellular products including proteins, RNA and new cellular DNA. Synchronized cells also regulate the synthesis of viral products. For example, mitosis is required before oncornavirus particles and their structural proteins can be detected (9). In lymphoid cells persistently infected with feline leukemia virus (FeLV), feline oncornavirus-associated cell membrane antigen (FOCMA) production was highest in the G1 phase of the cell cycle (10). Preliminary experiments showed that the absolute number of E and EAC-binding cells in two different lymphoid cell lines varied with the age and cell density of the cultures used. These data suggested that the expression of these cell markers might depend upon cell cycle kinetics. Accordingly, we examined the influence of cell synchronization and subsequent release in complete medium upon the detection of T and B cell receptors. Our results suggest that synchronization has a profound effect on the expression of these markers and, in fact, can affect the actual proportion of cells identified as T cells, B cells or nonrosetting null cells.

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MATERIALS AND METHODS

Cells. Two different continuous lines of lymphoid cells were employed. The CT45-S line originally derived by John Mitchell (Department of Public Health, 3500 North Logan Street, Lansing, Michigan 48914) was kindly supplied by Max Essex (Harvard School of Public Health, 665 Huntington Avenue, Boston, Massachusetts 02115). The cell was derived from a primary culture of normal canine thymus tissue but expresses only the EAC (B cell) surface marker. The FL-74 cell, a continuous cell line derived by Theilen (11) from a cat with an FeLV-induced lymphoid tumor, was obtained from Dr. Max Essex. This cell is infected with and sheds the Kawakami-Theilen strain FeLV and expresses the T-lymphocyte marker for the feline species (12). Using a membrane immunofluorescence assay, both CT45-S cells and FL-74 cells lack detectable surface immunoglobulin (S. Krakowka, unpublished observations).

Cultural conditions and cell synchronization. Cells were propagated in Falcon plastic flasks (75-cm²) as suspension cultures in McCoy 5A complete medium supplemented with antibiotics and 15% fetal bovine serum (10).

For cell synchronization, actively replicating cells, i.e. 2 logs less than saturation density, were harvested and washed twice in prewarmed isoleucine-deficient medium (Biolabs, Inc., 2910 MacArthur Boulevard, Northbrook, Illinois; lot no. 41217) supplemented with dialyzed fetal bovine serum and antibiotics and 15% fetal bovine serum (10)

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As described initially by Ley and Tobey (13) and subsequently applied to synchrony of FL-74 cells by Olsen et al., medium for 24 hr resulted in a viable cell population arrested in the G1 phase of the cell cycle. Following a synchronization treatment period of 24 hr, cells were washed and resuspended in triplicate in prewarmecl complete medium. At intervals thereafter, aliquots of ceils were evaluated for total cell numbers, viability as measured by trypan blue dye exclusion and expression of cell surface markers.

Enumeration of T cell rosettes. It has been previously shown that canine and feline T lymphocytes will rosette with guinea pig erythrocytes (12,14). Guinea pig blood was drawn on the day of the experiments and the erythrocytes (E) obtained were treated with neuraminidase (General Biochemicals, Chagrin Falls, Ohio; lot no. 44328; 40 µ per ml) for 1 hr at 37°C. Concentrations of neuraminidase-treated E and lymphocytes were adjusted to a 30 : 1 ratio in V-bottom hemagglutination plates (microtiter disposable plates; Cooke Engineering, Alexandria, Virginia), incubated at 37°C for 15 min, centrifuged at 200 × g for 2 min and subsequently allowed to remain indisturbed at room temperature (22°C) for 1 hr. For counting, 1 drop of new methylene blue (0.05% in saline) or 0.5% trypan blue in saline was added and an aliquot of the suspension transferred to a hemacytometer chamber. Cells with three or more attached E were considered positive rosettes.

Enumeration of B cell rosettes. The erythrocyte-antibody-complement (EAC) rosette assay currently in use in this laboratory has been described previously (12). Briefly, sheep erythrocytes (E) were preincubated with a subagglutinating dose of canine origin IgM anti-sheep E serum. Subsequently, an equal volume of mouse complement prediluted 1 : 10 in isotonic sodium barbital buffer was added to the mixture and incubated an additional 30 min at 37°C. The EAC complex formed was washed in Hanks' balanced salt solution (HBSS) and resuspended to a cell concentration of 3 × 10⁷ cells per ml. Aliquots of lymphocytes were incubated with EAC in V-bottom plates at 30 : 1 ratio for 1 hr at 37°C. EAC-rosettes were enumerated in a hemacytometer chamber in a similar manner as described for T rosettes. Controls for nonspecific rosette formation with sheep E coated with antibody (EA) were included with each test.

Separation of rosetting from nonrosetting CT45-S cells. Five ml of CT45-S cells (1 × 10⁶ cells per ml) were incubated with 0.5 ml of sterile EAC for 1 hr at 37°C. The suspension was then diluted to 20.0 ml with fresh complete medium and overlaid on 15.0 ml of ficoll-hypaque (specific gravity 1.0805). Following centrifugation at 400 × g for 40 min, cells at the ficoll-hypaque interface and in the pellet were examined for rosettes, washed, resuspended, and restested for their ability to form EAC and then seeded into Linbro plates (60 by 15 mm; Linbro Chemical Co., New Haven, Connecticut) in complete medium for propagation at a cell concentration of 1 × 10⁶ cells per ml. Aliquots were tested at intervals thereafter for rosette formation with EA and EAC.

Effects of old medium on the enumeration of rosettes. Cell-free culture medium from cells seeded 6 days previously was clarified by centrifugation at 1000 × g for 10 min. Freshly pre-