Relation of gp70 to Spontaneous Cytolytic Activity of Mouse Spleen Cells

Antoinette Hatzfeld, Abraham Pinter, Gloria C. Koo, and Edward A. Boyse
Memorial Sloan-Kettering Cancer Center, 1275 York Avenue, New York, New York 10021

Abstract. In comparing spleen cells of inbred and congenic mice for spontaneous capacity to lyse cells of the BALB/c leukemia RL\_31 in vitro, we found that the activity of 129 spleen cells was more than double that of 129-G\_ix\^- spleen cells. The only known difference between these two strains is that 129-G\_ix\^- mice express no known demonstrable gp70 or p30, whereas 129 mice express both these MuLV-related components as mendelian traits not associated with the production of virions. We infer that MuLV-related components at the cell surface are concerned in effector-target interactions leading to cytolysis under the conditions described. — Although the congenic strains B6 (G\_ix\^-) and B6-G\_ix\^+ differ likewise in expression of the type-variant G\_ix-gp70, both strains express a second type-variant of gp70. The lytic activity of spleen cells of these two strains for RL\_31 cells was equally high, suggesting that involvement in lytic effector-target interactions is common to gp70 molecules in general. — When used as targets rather than as effectors 129 spleen cells were more sensitive to lysis than 129-G\_ix\^- spleen cells. Pre-exposure to gp70, purified from R-MuLV, rendered splenic effector cells less lytic. Pre-exposure to gp70 also rendered RL\_31 target cells less sensitive to lysis. One explanation of these findings is that both target cells and effector cells express gp70 and also receptors for gp70 and that this is the basis of mutual cellular recognition leading to lysis in the circumstances described.

Introduction

In comparing strains of inbred and congenic mice for the spontaneous capacity of their cells to lyse cells of the leukemia BALB.RL\_31 (RL\_31) in vitro, we consistently found more lysis by 129 strain spleen cells than by congenic 129-G\_ix\^- strain spleen cells.

The only known genotypic difference between these congenic strains is at the Ge-I locus (Stockert et al. 1975), which determines expression versus nonexpression...
of G\textsubscript{ix}-gp70, a type-variant of the gp 70 family of molecules that form the envelopes of C-type viruses (Tung et al. 1975). Neither 129 nor 129G\textsubscript{ix} mice are overt producers of C-type viruses, but 129 mice express G\textsubscript{ix}-gp70 (Stockert et al. 1971), and also the p30 internal protein of C-type virus (Strand et al. 1974), whereas 129-G\textsubscript{ix} mice express no known variety of gp70 (Stockert et al. 1975, Tung et al. 1975), or p30, (Strand et al. 1974).

Accordingly, levels of lytic activity against RL\textsubscript{5}1 cells evidently depend on whether the mouse donating the effector cells expresses MuLV-related proteins, which may therefore be involved in recognition between effector cells and target cells in this cytolytic system, as has been suggested by others in similar circumstances (Kende et al. 1979).

Materials and Methods

Mice

All mice were bred in colonies maintained at Sloan-Kettering Institute. The mice in each experiment were closely matched for age (4-8 weeks), but not necessarily for sex, because in regard to lytic activity in the assay described below, we found no significant difference between the sexes at any age.

Reagents

RPMI (Gibco) was supplemented with fetal calf serum (10% for culture, 5% for cytolytic assay), glutamine (5 mM), penicillin (100 U/ml) and streptomycin (100 \mu g/ml).

Bovine serum albumin (BSA) was purchased from Miles Laboratories, Inc., and fetuin (from fetal calf serum) B grade from Calbiochem, California.

Viral proteins

Rauscher virus and AKR virus were supplied by the National Cancer Institute. Purified gp70 and p30 were prepared from Rauscher virus by ion exchange chromatography according to Strand and August (1973) and evidence of purity is given in Figure 1.

Cytolytic assay

Target cells (T). RL\textsubscript{5}1 ascites leukemia cells (Sato et al. 1973) were harvested from passage in the strain of origin, BALB/c, and cultured in supplemented RPMI. After not less than 2 weeks, cells were prepared for assay by culture for 24 h in supplemented RPMI at an initial density of $2 \times 10^5$ per ml then labelled with $^{51}$Cr for 1 h at 37\degree C (100 \mu Ci per $8 \times 10^6$ cells in 0.5 ml supplemented RPMI). Spleen cells, for use as targets, were labelled in the same way (300 \mu Ci per $30 \times 10^6$ cells) without preliminary culture.

Effector cells (E). Spleen cells were fractionated on a BSA density gradient as described and illustrated by Scheid and co-workers (1978). Layers A and B were pooled and the cells washed three times.

Procedure. $^{51}$Cr release assay in microtiter plates was performed according to Canty and Wunderlich (1970). For the standard cytolytic assay (in triplicate), effector cells were mixed with labelled target cells in a total volume of 80 \mu l, and incubated on a rocking platform for 6 h in a humidified atmosphere with 10% CO\textsubscript{2}. As a control for $^{51}$Cr release unrelated to the cytolytic activity under study, effector spleen cells were replaced by unlabelled RL\textsubscript{5}1 cells (control B below).

Supernatants were collected with Titertek apparatus (Flow Laboratory). Results are expressed as "percent lysis" of target cells, calculated from: $100 (A-B)/(C-B)$ where A = cpm experimental, B = cpm control B (see above), and C = cpm from lysis of hydrochloric acid.

Target cells or effector cells were exposed to gp70 and other agents (indicated in Figure 2 and in the text) for 5 min at room temperature, and were washed twice before assay.