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Immunology

Expression of endogenous retroviral glycoprotein 70 by antigen-activated cytotoxic and suppressor T lymphocytes of mice

(differentiation/viral gene expression/membrane antigen/type C virus)

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ABSTRACT **Concanavalin A-stimulated murine spleen cells and antigen-stimulated B lymphocytes of normal mice express an antigen that reacts with goat antiserum against glycoprotein (gp) 70. Structural analysis of this antigen characterizes it as endogenous viral gp70 that is most likely of xenotropic origin. Activated nonspecific T suppressor cells and cytotoxic T lymphocytes express endogenous viral gp70, whereas nonactivated mouse T or B lymphocytes do not. The presence of endogenous retroviral gp70 is thus a novel marker for activated mouse lymphocytes in general.**

The major viral envelope glycoprotein, gp70, of murine type C viruses (1) is also found in the cell membrane of virus-producing cells (2). It has been suggested that the expression of gp70 might be linked to processes of cellular growth or differentiation (or both) (3, 4). The synthesis of endogenous type C viruses has been demonstrated in mouse lymphocytes after activation by mitogens or alloantigens during the graft-vs.-host (GvH) reaction (5, 6). Viral gp70 can be expressed even in the absence of overt virus expression (7).

We have reported previously the appearance of an antigen resembling endogenous viral gp70 in membranes of mouse T helper and B cells that were activated by mitogens (8) or antigens (8, 9). This antigen was characterized serologically by using antisera prepared against purified Friend leukemia virus (FLV) gp70 and feline leukemia virus (10).

We report here (*i*) that this antigen resembles a type C virus-specific glycoprotein with a M_r of 70,000 and (*ii*) that it can be detected in the membranes of antigen-activated cytotoxic T cells and nonspecific T suppressor cells of mice.

MATERIALS AND METHODS

Antisera. Goat anti-FLV-gp70 antiserum was a gift from Werner Schäfer, MPI für Virusforschung, Tübingen, West Germany (10). The gamma globulin fractions were prepared by consecutive precipitation with 50% and 30% ammonium sulfate followed by dialysis. Anti-Thy 1.2 monoclonal antibody was a gift from Bayer AG (Elberfeld, West Germany).

Treatment of Cells with Antisera. Spleen cells were incubated with either a 1:5 dilution of the anti-gp70 gamma globulin fraction (2×10^7 cells) or a 1:1000 dilution of monoclonal anti-Thy 1.2 (6×10^7 cells) for 30 min on ice. After sedimentation, the cells were suspended in 1 ml of appropriately diluted rabbit complement absorbed with agarose and tested for low mouse lymphocyte toxicity.

Induction of Suppressor Cells. The method described by Pickel and Hoffman (11) was modified as follows: 6 to 7×10^7 spleen cells of C57BL/6 mice were injected intravenously into (C57BL/6 × DBA/2)F_1 mice. After 12 days, the spleen cells of the recipient mice were obtained. The spleen cell suspensions and *in vitro* culture system have been described by Mishell and Dutton (12).

***In Vitro* Induction of Cytotoxic T Lymphocytes (CTL).** Alloreactive CTL were generated by incubating 2×10^7 DBA/2 spleen cells with 1×10^7 semiallogeneic spleen cells of (CBA × DBA/2)F_1 mice for 4 days. The cells were then used for ^{51}Cr release assay. *In vitro* induction of CTL by syngeneic haptenated stimulator cells was as described by Shearer (13). Briefly, 2×10^7 spleen cells of (CBA × DBA/2)F_1 mice were incubated with 4×10^6 syngeneic trinitrophenyl (TNP)-modified or normal spleen cells for 5 days. The cells were then washed and used for ^{51}Cr release assays with appropriate target cells.

Labeling of Target Cells. Monolayers of mouse L cells (syngeneic with CBA, allogeneic with DBA/2) in 50-ml culture flasks were incubated with 2 ml of RPMI 1640 medium/2% fetal calf serum containing 100 μCi (1 Ci = 3.7×10^{10} becquerels) of $Na^{51}CrO_4$ (specific activity, 200–500 Ci/g of chromium) for 1 hr at 37°C on a rocker platform. Then, the cell layers were washed three times with balanced saline solution/2% fetal calf serum and trypsinized. The suspended cells were then incubated with TNP sulfonic acid as described by Shearer (13) to obtain haptenated syngeneic targets or washed three more times and used as such for allogeneic targets. The ^{51}Cr release assay (13) was carried out in triplicate using round-bottom microtiter plastic plates. Target cells (1×10^4) in 100 μl of RPMI 1640 medium/5% fetal calf serum were seeded into each well. The *in vitro*-activated spleen cells were added in 100 μl of medium to give effector/target ratios of 40:1, 20:1, and 10:1. Positive controls received 100 μl of 1% Triton X-100 solution and spontaneous-release controls received 100 μl of medium instead of the effector cell suspensions. After 5 hr of incubation at 37°C, 100 μl of supernatant was withdrawn from each well for assay in a γ-ray counter. Results are expressed as % specific lysis = [(cpm experimental − cpm spontaneous)/cpm positive control − cpm spontaneous)] × 100.

Cell-Surface Labeling and Immunoprecipitation. Cells were washed twice with phosphate-buffered saline and then suspended in phosphate-buffered saline/5 μM KI. Lactoperoxidase-catalyzed iodination was carried out as described (14). Labeled cells were washed, extracted, and immunoprecipitated with normal or immune serum according to White *et al.* (15). $NaDodSO_4$/polyacrylamide gel electrophoresis was carried out according to Laemmli (16).

Peptide Mapping. ^{125}I-Labeled gp70 bands were localized by autoradiography, extracted from the gel, and analyzed as described by Hutchinson *et al.* (17). Enzyme digestion was car-

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Abbreviations: gp, glycoprotein; GvH, graft vs. host; FLV, Friend leukemia virus; CTL, cytotoxic lymphocytes; TNP, trinitrophenyl.

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ried out for 8 hr at 37°C with *N*-tosylphenylalanine chloromethyl ketone/trypsin (Worthington) at 60 μg/ml.

RESULTS

Structural Analysis of gp70 in Membrane of Concanavalin A-Stimulated Mouse Spleen Cells and Nylon Wool-Purified T Cells. The gp70 of murine type C viruses is linked by disulfide bridges to polypeptide p15(E) which, in turn, is anchored in the cellular membrane (18). The ^{125}I-surface-labeled and immunoprecipitated complex forms a band at M_r 85,000 under nonreducing conditions when assayed by polyacrylamide gel electrophoresis (Fig. 1). In the presence of 2-mercaptoethanol, however, the disulfide bridges are cleaved and the labeled glycoprotein shows its characteristic M_r of 69,000–71,000; polypeptide p15(E) remains unlabeled under these conditions. Exactly this behavior is exhibited by the material precipitated by our anti-FLV-gp70 antiserum from Nonidet P-40 extracts of C57BL/6 mouse spleen cells activated with concanavalin A and peroxidase iodinated. The same holds true if nylon wool-purified T cells are used instead of unseparated spleen cells. This is of interest because concanavalin A, although primarily a T-cell mitogen, can also stimulate mouse B cells if aided by a concanavalin A-induced (supernatant) factor produced by T cells (19).

Tryptic peptide maps of gp70s derived from concanavalin A-stimulated C57BL/6 mouse spleen cells and from AKR virus-producing cells are shown in Fig. 2. The patterns of gp70 from C57BL/6 cells and from the ecotropic virus are definitely similar, but there are also differences that suggest a xenotropic origin (14).

These results demonstrate the similarity of the antigen on the surface of concanavalin A-activated cells to viral glycoprotein and strongly suggest its association with an endogenous viral locus.

Gp70 on the Surface of Activated Non-Antigen-Specific Suppressor Cells. Suppressor cells present in mouse spleen 12 days after induction of a GvH reaction (11) completely suppress *in vitro* antibody responses to sheep erythrocytes by spleen cell cultures derived from such mice. Strong suppression is also seen in 90% syngeneic normal spleen/10% GvH-induced spleen cell mixtures. This is shown in Table 1, experiment 1. However, treating the GvH-induced spleen cells with goat anti-gp70 serum/complement before adding them to syngeneic indicator cultures removes suppressive activity. Treatment with either normal goat serum/complement or complement alone had no such effects.

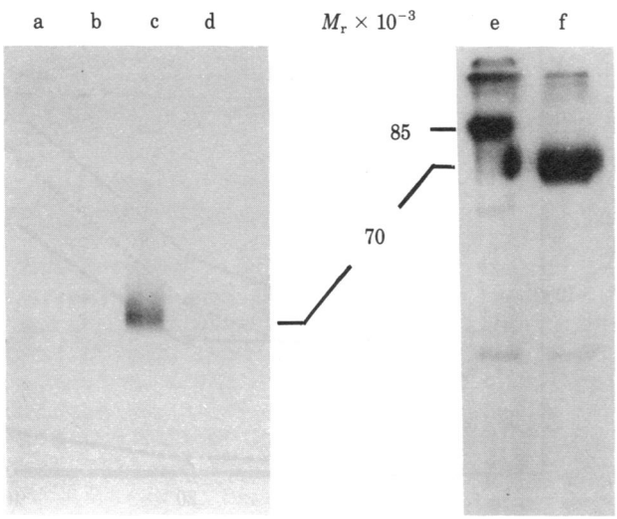

FIG. 1. Electrophoretic analysis of ^{125}I-labeled immunoprecipitates from control (lanes a and b) and concanavalin A-stimulated (lanes c–f) C57BL/6 spleen cells. Lanes: b and d, precipitates with normal goat serum; a, c, e, and f, precipitates with anti-FLV-gp70 serum. The sample in lane e was not treated with 2-mercaptoethanol.

The high degree of specificity of the antiserum in recognizing and eliminating only the activated suppressor cells is shown in Table 1, experiment 2. In that case, the cultures contain only GvH-induced spleen cells and, as such, are not able to produce any anti-sheep plaque-forming cells, (PFC), being totally suppressed. Treatment of these spleen cells with anti-gp70 antiserum/complement before culture results in a perfectly normal anti-sheep response. The phenotype of the suppressor cells in these experiments was determined to be Thy 1^+ Lyt 1, 2, 3^+, in accordance with what has been described in the literature for GvH-induced suppressors (11).

Thus, the activated lymphocytes (i.e., the T suppressor cells) are preferentially if not exclusively eliminated by anti-gp70 treatment whereas none of the sheep-reactive T helper and B precursor cells are affected. We have previously shown, however, that, after antigen stimulation, T helper and B cells are also recognized by the same antiserum and are lysed in the presence of complement (8, 9). Thus, the activation of mouse B, T helper and T suppressor precursor cells by either antigens or mitogens seems to be a prerequisite for the expression of endogenous type C viral gp70.

Gp70 on the Surface of Activated Cytotoxic T Cells. Because only the humoral immune system had so far been implicated in the expression of endogenous viral gp70 on lymphocyte surfaces (8, 9, 20), we investigated whether or not CTL also express gp70. CTL were generated *in vitro* by activation with semiallogeneic stimulator cells in a one-way mixed lymphocyte culture (21) and by activation with syngeneic haptenated stimulator cells (13).

The results with the first system are shown in Fig. 3. At an effector/target cell ratio of 40:1, ≈20% specific lysis of the target cells was observed. Similar results were obtained with cytotoxic effector cells previously treated with complement alone or in the presence of normal goat serum. Treatment with anti-gp70 antiserum/complement, however, drastically reduced specific lysis, indicating that alloantigen-activated cytotoxic cells do express gp70. These cells were also completely removed by treatment with anti-Thy 1.2/complement and thus identified as CTL.

Similar results were obtained with the second system (Fig. 4). At an effector/target ratio of 40:1, 50% specific lysis of TNP-haptenated syngeneic targets was observed and none of the control treatments had any demonstrable effect. Anti-gp70/complement again significantly reduced cytotoxic activity, albeit not as completely as in the first system. Sensitivity to treatment with anti-Thy 1.2/complement again characterized the gp70-positive effector cells as CTL.

DISCUSSION

Mouse genomes contain a multitude of integrated viral sequences, most of which are defective (22, 23). The expression of gp70 is not always associated with the coordinate synthesis of other structural viral components or indeed with virion synthesis (7). Therefore, the reported failures to induce the production of type C virus particles by alloantigenic or mitogenic stimulation in lymphocytes of all mouse strains or with any mitogen does not imply the total absence of inductive processes (24). We have found that the expression of gp70 can be induced by seemingly normal antigenic stimulation in B lymphocytes of all mouse strains tested, including those that are reportedly negative. The same is true for T helper cells and for nylon wool-

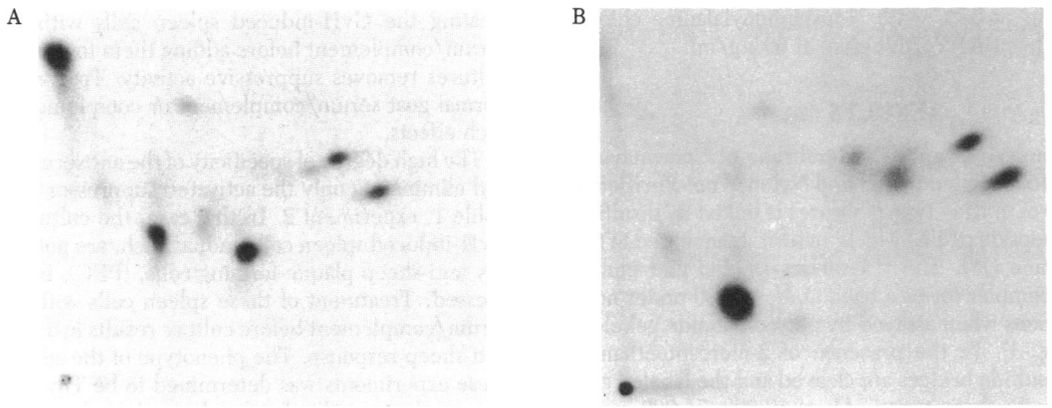

FIG. 2. Tryptic peptide maps of ^{125}I-labeled gp70s from concanavalin A-stimulated C57BL/6 spleen cells (*A*) and from AKR virus-producing cells (*B*). Two-dimensional analysis was carried out as described (17); electrophoresis was from left to right.

separated T lymphocytes after stimulation with concanavalin A.

Schumann and Moroni (24) were unable to induce the synthesis of type C virus in T lymphocytes treated with concanavalin A. In the absence of complement, their antiserum, which was specific for glycopolysaccharide-induced BALB/2 endogenous xenotropic virus, had no effect on a mixed lymphocyte culture or on the generation of CTL. However, it did inhibit humoral immune responses *in vitro*. The antiserum used in our study was prepared in goats against purified FLV gp70 and is specific for gp70. In the absence of complement, it had no effect on mouse lymphocytes. The serum used by Moroni and coworkers also reacted with major viral structural antigens other than gp70. Thus, the discrepancies between our results and theirs are probably due to the different specificities of the antisera used.

Our results show that CTL and non-antigen-specific T suppressor cells of mice contain endogenous viral glycoproteins in their membranes. The expression of viral gp70 seems to be characteristic of activated lymphocytes and is thus a specific marker for activated murine lymphocytes in general. Treatment of GvH-induced spleen cultures with anti-gp70/complement fully restores the plaque response that is otherwise absent due to non-antigen-specific T suppressor cells (Table 1). Thus, the resting sheep antigen-responsive T and B precursor cells present in the spleen cell population are not at all affected by treatment that eliminates the activated suppressor cells. Furthermore, the resting precursors of suppressor cells express viral gp70 either not at all or in undetectable amounts. Similarly, England and Halpern found endogenous RAV-O antigens in clusters of splenic B lymphocytes only after immunological sensitization of chickens (25).

Our findings are particularly interesting in the context of the recently described long terminal repeat structures on either end of integrated retroviral genomes (26–28). The long terminal repeats of both avian and murine exogenous proviruses possess promoter activities that, possibly depending on the site of the integration, can be modified by cellular restraints. In other instances, however, the viral promoters lead to constitutive transcription and, ultimately, to the expression of viral and adjacent cellular genes (29–33). Endogenous proviruses also have long terminal repeat sequences (J. N. Ihle, personal communica-

Table 1. Specific elimination of nonspecific T suppressor cells by treatment with anti-gp70 antiserum/complement

Spleen source	Cells in culture, no.	Treatment before culture	pfc per 10^6 IgM, no.
	Experiment 1		
GvH	5×10^6	None	0
$B_6D_2F_1$	5×10^6	None	1396
$B_6D_2F_1$/GvH	$5 \times 10^6/5 \times 10^5$	None	19
$B_6D_2F_1$/GvH	$5 \times 10^6/5 \times 10^5$	anti-gp70/RC*	1033
$B_6D_2F_1$/GvH	$5 \times 10^6/5 \times 10^5$	NGS/RC*	15
$B_6D_2F_1$/GvH	$5 \times 10^6/5 \times 10^5$	RC*	7
	Experiment 2		
GvH	5×10^6	None	0
$B_6D_2F_1$	5×10^6	None	1651
GvH	5×10^6	anti-gp70/RC	1917
GvH	5×10^6	NGS/RC	320

pfc, Plaque-forming cells; $B_6D_2F_1$, (C57BL/6 × DBA/2)F_1; RC, rabbit complement; NGS, normal goat IgG.
* Only GvH cells were treated before addition to cultures.

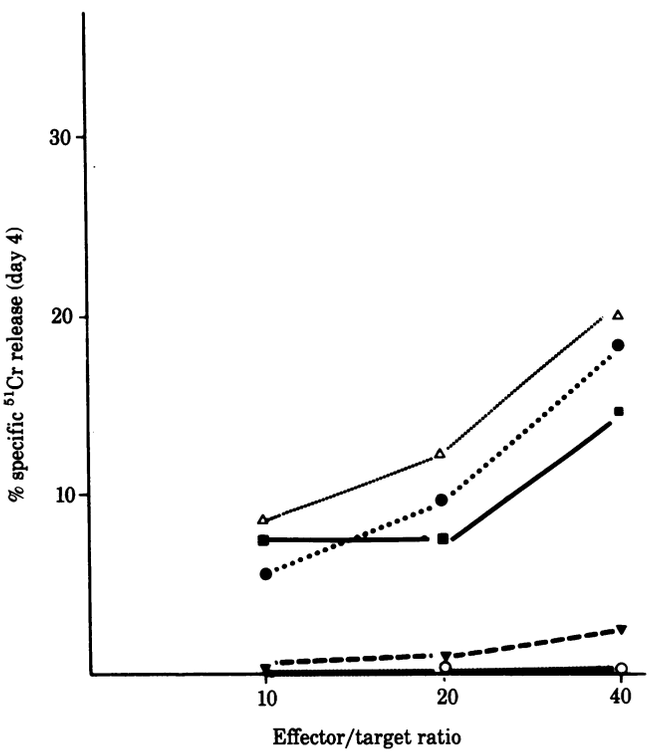

FIG. 3. Effect of treatment with anti-gp70 antiserum/complement on alloreactive CTL of DBA/2 mice. △, Rabbit complement alone; ●, control; ■, normal goat IgG/complement; ▼, goat anti-gp70 IgG/complement; ○, monoclonal anti-Thy 1.2/rabbit complement.

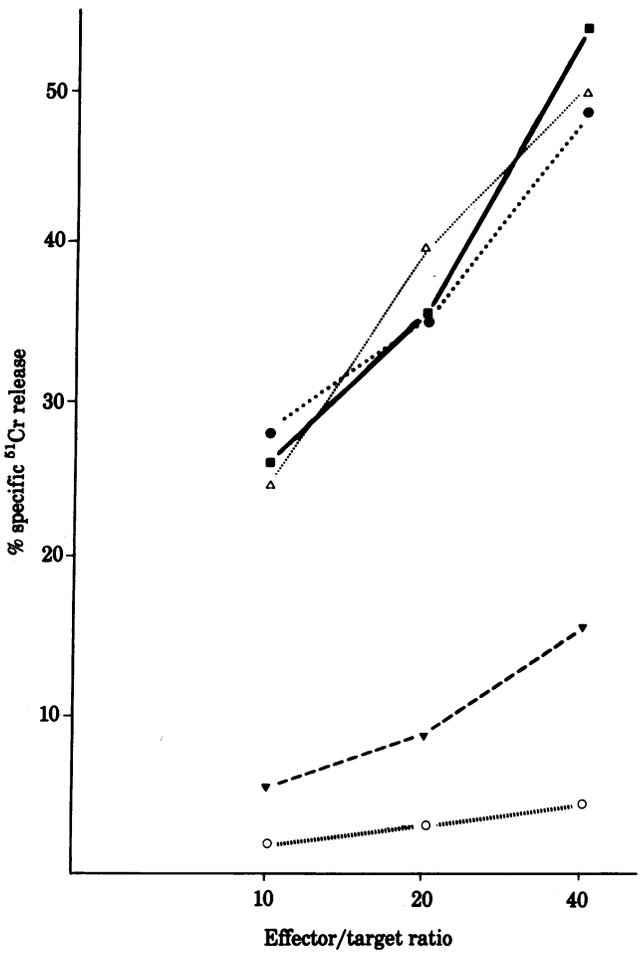

FIG. 4. Effect of treatment with anti-gp70 antiserum/complement on TNP-specific CTL of (CBA × DBA/2)F_1 mice. ■, Normal goat IgG/rabbit complement; △, rabbit complement alone; ●, control; ▼, goat anti-gp70 IgG/rabbit complement; ○, monoclonal anti-Thy 1.2/rabbit complement.

tion), and any such promoter activity, but in particular that of xenotropic proviruses, seems to be under negative cellular control in resting lymphocytes as well as in most other murine tissues (34). The cellular restraints on endogenous viral gene expression were shown to be released by the addition of nucleoside analogues such as 5-bromodeoxyuridine and protein synthesis inhibitors (24, 35–37). The inducibility of endogenous viral gene expression described by us represents, in terms of viral gene regulation, one of the most consistent models available and it does not depend on nonphysiological factors such as 5-bromodeoxyuridine. Moreover, it provides a novel and suitable system to study cellular gene regulation in murine lymphocytes.

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