Bone Marrow–Derived Lymphoid Cells (B Cells): Functional Depletion with Cobra Factor and Fresh Serum

Abstract. *Treatment of rat spleen cells with cobra factor and fresh rat serum provided a simple, rapid means of functionally eliminating complement receptor lymphocytes. Cells able to differentiate into plaque-forming cells in a syngeneic, irradiated host were diminished, but cells able to induce a graft-versus-host reaction were not diminished. There was no effect on plaque-forming cells from an immune spleen.*

Attempts have been made to bolster tumor patients' immune anticancer defenses by incubation in vitro of their lymphoid cells with autologous tumor cells, and then reinoculating the lymphocytes (*1*). Such attempts may be complicated by the simultaneous transfer, in the treated lymphoid cells, of not only cytotoxic lymphocytes—presumably thymus-derived (T) cells (*2*)—but also immune marrow-derived (B) lymphocytes, which may be producing blocking factors (*3*) deleterious to the host. We have developed a simple procedure to eliminate functionally from a lymphoid cell population at least part, if not all, of the B cells.

It is well established that a factor in cobra venom (CoF), in concert with heat-labile normal serum proteins, is able to activate the terminal complement components, C3 to C9 (*4*). Such activation of C3 through C9 by CoF in the presence of unsensitized erythrocytes can result in passive hemolysis of these cells (*5*). Bianco *et al.* (*6*) have shown that at least some B cells have surface receptors for C3. We, therefore, sought to determine whether these complement receptor lymphocytes (CRL) would be particularly susceptible to passive uptake of complement that had been activated by CoF. We now report on the functional depletion of CRL from lymphoid cells incubated with CoF-activated serum. Elimination of CRL diminished the number of cells able to differentiate into plaque-forming cells (PFC) in a syngeneic irradiated host, but had no effect on that population of lymphoid cells responsible for the graft-versus-host reaction. This procedure, however, did not reduce the number of PFC already in an immune spleen.

Nonimmune spleen cells from inbred Lewis rats (Microbiological Associates) were washed twice with cold medium 199 containing 5 percent heat-inactivated fetal bovine serum (M199/FBS) (Grand Island Biological). Immune spleen cells were obtained from Lewis rats immunized 5 days earlier with 1 ml of a 2 percent suspension of thrice-washed sheep red blood cells. In experiments on the effect of CoF-activated serum on CRL from nonimmune rats and on PFC from animals immune to sheep red blood cells, 2.5×10^7 washed nonimmune and immune spleen cells, respectively, were suspended in 1 ml of serial dilutions of fresh rat serum (*7*), each dilution containing 0.1 ml of undiluted CoF (*8*), and incubated at 37°C with vigorous shaking for 60 minutes. Control cells received serial dilutions of heat-inactivated (45 minutes at 56°C) rat serum plus CoF. Cells were washed four times with M199/FBS, and the nonimmune cells were assayed for CRL by the method of Bianco *et al.* (*6*), in which sheep red blood cells bearing C3 were prepared by incubating sensitized red blood cells with a 1 : 10 dilution of fresh mouse serum. The immune spleen cells were assayed for PFC by a modification of the method of Jerne *et al.* (*9*).

The nonimmune spleen cells used for adoptive transfer and graft-versus-host experiments were treated as above in a 1 : 3 dilution of fresh or heat-inactivated rat serum containing 10 percent undiluted CoF and washed four times. Portions containing 11×10^6 cells were injected intravenously into six syngeneic Lewis rats exposed 24 hours previously to 600 roentgens of ^{60}Co γ-radiation. The rats were then immunized with 1 ml of a 2 percent suspension of sheep red blood cells, and the Jerne assay was performed 7 days later. Additional portions of treated and control nonimmune spleen cells containing 4.4×10^6 cells were washed and injected into a hind footpad of six 3- to 4-week-old LBN F_1 recipients. After 8 days, the popliteal nodes from the injected and contralateral legs were removed and weighed. The graft-versus-host reaction so induced (*10*) was quantified as the ratio of the weight of the draining lymph node to that of the contralateral lymph node.

Figure 1 shows the effect of serial dilutions of CoF-activated rat serum on the percentage of depletion of CRL from normal rat spleen cells and of PFC from immune rat spleens. The maximum effect on CRL was seen with a 1:5 serum dilution, which reduced by more than 70 percent the CRL fraction of the total nucleated cells when compared to cells treated with heat-inactivated rat serum and CoF. The lesser effect of undiluted rat serum may be due to natural complement inhibitors found in low concentration in normal serum (*11*). Unlike with CRL, CoF-activated fresh serum had no effect on the number of PFC in the spleens of rats already immune to sheep

Table 1. Effect of CRL depletion on the ability of rat spleen cells to induce a graft-versus-host (GVH) reaction and, after adoptive transfer, to differentiate into plaque-forming cells (PFC). Lewis rat splenocytes were incubated at 37°C for 60 minutes with a 1 : 3 dilution of either fresh or heat-inactivated rat serum containing 10 percent undiluted cobra factor (CoF). One portion of cells (4×10^6) were injected into one footpad of each of six LBN F_1 rats. Draining (D) and contralateral (C) popliteal lymph nodes were weighed 8 days later. The GVH is expressed as the ratio of the weight of D to C. Another portion of the cells (11×10^6) was injected intravenously into six syngeneic irradiated rats that were then immunized with sheep erythrocytes. Spleens were assayed for direct PFC 7 days later.

Serum + CoF	GVH reaction (D/C ± S.E.)	Adoptive transfer (PFC/spleen ± S.E.)*
Fresh	5.1 ± 0.9	3326 ± 1308
Heated	4.9 ± 1.4	10413 ± 2078

* Similar results were obtained when data were expressed as PFC/10^6 spleen cells; $.01 < P < .02$.

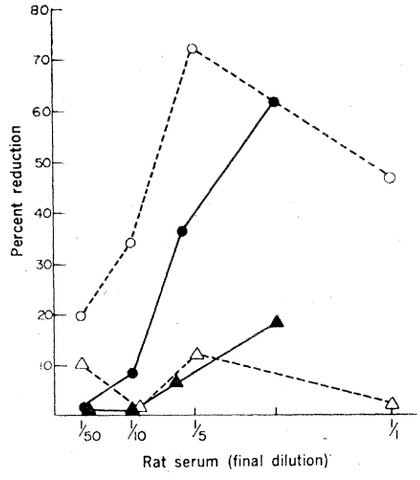

Fig. 1. Effect of cobra factor and fresh rat serum on complement receptor lymphocytes (circles) and plaque-forming cells (triangles). Dashed and solid lines denote two experiments. The results are expressed as the percentage reduction of controls treated with cobra factor and heat-inactivated rat serum.

erythrocytes. This is consistent with the report (*6*) that antibody-forming cells do not bear C3 receptors.

Rat serum activated by CoF diminished by threefold the ability of normal rat spleen cells to differentiate into hemolytic plaque-forming cells in syngeneic irradiated hosts challenged with sheep red blood cells (Table 1). The same treated cells were unimpaired in their ability to induce a graft-versus-host reaction in semiallogeneic LBN rats (Table 1), suggesting that CoF-activated serum had no effect on T lymphocytes.

Other procedures have been used to eliminate B cells (*6, 12*). Differential centrifugation and treatment on columns containing immunoadsorbents—such as immune complexes or antibody to immunoglobulins—may be effective methods, but are quite laborious. The specific lysis of B cells by antibody to immunoglobulin and complement may be obscured by the presence of some immunoglobulin determinants on T cells (*13*). Our procedure is simple and rapid; it can, in theory, be used on the lymphoid cells from any species. Indeed, our recent experiments have shown it effective with both mouse and human cells (*14*).

While it is interesting to postulate that CRL were destroyed by passive cytolysis in vitro, two other possibilities must be considered. Cells coated with C3 may be more susceptible to phagocytosis in vivo, or these cells may be sterically hindered from performing their function by cell-bound complement components. Whether all B cells are affected by our procedure and whether the effects totally spare T cells remain to be determined.

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7. Pooled rat serum [44 hemolytic complement (CH_{50}) unit/ml] was obtained from Suburban Serum Laboratories, Silver Spring, Maryland. It contained no measurable cytotoxic activity against Lewis rat spleen cells.
8. For separation of cobra factor (CoF), lyophilized *Naja siamensis* venom (Miami Serpentarium) was placed on a DEAE cellulose column (100 by 2.5 cm) equilibrated with 0.005*M* phosphate buffer (*p*H 7.4); the column was eluted with a continuous salt gradient (0.005 to 0.5*M* NaCl) in 0.005*M* phosphate buffer. The CoF activity appeared in the second protein peak and, at a 1 : 10,000 dilution, diminished by greater than 50 percent the CH_{50} activity of whole guinea pig serum (188 CH_{50} unit/ml), but was nontoxic when injected undiluted into 4-day-old mice.
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Effect of Colchicine, Colcemid, and Vinblastine on the Agglutination, by Concanavalin A, of Transformed Cells

Abstract. *The sensitivity of transformed 3T3 fibroblasts to agglutination by concanavalin A is reduced by alkaloids that bind specifically to protein subunits of microtubules.*

Tumor cells that have been transformed by viruses or other agents develop a sensitivity to agglutination by concanavalin A (Con A) and some other lectins (specific proteins that bind carbohydrates) (*1*). The increased agglutinability appears not to reflect an increase in the number of Con A binding sites (*2*), but rather a change in their surface topography: a shift from a normal homogeneous or random pattern to a heterogeneous or patchy distribution (*3*). In an attempt to determine the factors that facilitate agglutination, or effect the segregation of binding sites, or both, we have examined the agglutination, by Con A, of polymorphonuclear leukocytes (PMN) (*4*). These normal cells have Con A binding sites distributed heterogeneously over their surfaces (*5*), and are agglutinable by Con A. Low concentrations of colchicine and vinblastine, alkaloids that bind to protein subunits of microtubules, reduced the sensitivity of PMN to agglutination. We report here the effect of these alkaloids on the agglutination, by Con A, of the mouse fibroblast 3T3, and of 3T3 cells transformed by simian virus 40 (SV3T3). The characteristics of these cells are the prototypes of the above-mentioned differences between normal and transformed cells with respect to agglutinability and surface topography. We will show that colchicine, Colcemid, and vinblastine reduce the sensitivity of SV3T3 cells to agglutination by Con A.

The 3T3 and SV3T3 cells (*6*) were grown to confluence in plastic tissue culture flasks (Falcon) in Dulbecco's modified Eagle's medium (Grand Is-

Table 1. Effect of colchicine on the adherence of RBC's to 3T3 and SV3T3 cells treated with Con A. Nonconfluent monolayers of fibroblasts were incubated for 1 hour at 37°C with or without alkaloids in culture medium, washed three times in phosphate-buffered saline (PBS), incubated for 10 minutes with Con A (Miles-Yeda), and washed three times in PBS. A washed suspension of human type O outdated RBC's from a blood bank, at a hematocrit of 2 percent, was then layered over the monolayers and incubated for 20 minutes. The monolayer was then rinsed thoroughly with PBS, and fixed with 1 percent glutaraldehyde for 2 minutes. The adherent RBC's were enumerated under phase contrast microscopy. Colchicine was added at a concentration of $10^{-6}M$.

Treatment	Mean number of adherent RBC's per fibroblast* at Con A concentrations (μg/ml) of:					
	0	20	40	100	300	500
			3T3 Cells			
None	0.0	—	—	0.42	0.32	—
Colchicine	0.0	—	—	0.24	0.10	—
			SV3T3 Cells			
None	0.0	0.20	1.33	2.43	3.56	3.48
Colchicine	0.0	0.16	0.13	0.31	0.19	0.36

* Numerical index for hemagglutination was obtained by adding the total number of adherent RBC's, and dividing by the numbers of fibroblasts counted in random fields. When the numbers of RBC's adherent per fibroblast exceeded four (often in clusters), precise enumeration was difficult. Consequently, an arbitary value of ten RBC's per fibroblast was assigned for purposes of calculation.