SPECIFIC ELIMINATION OF CYTOTOXIC EFFECTOR CELLS

I. Adsorptive Behavior of Effectors and Their Precursors on Spleen Cell Monolayers

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Clonal diversity among T lymphocytes reactive to allogeneic transplantation antigens may be inferred from several lines of evidence. Antigen-dependent proliferation and uptake of heavily radiolabeled nucleic acid precursors by stimulated lymphocytes has been found to result in specific nonresponsiveness presumably in consequence of clonal deletion ("suicide") (1). Similar unresponsiveness was obtained after uptake of 5-bromodeoxyuridine and photoinactivation (2). Recently it has been possible to visualize directly receptor-bearing lymphocytes in unsensitized populations with anti-idiotypic sera which are presumably reactive with specific immune receptors for transplantation antigens (3, 4). Such anti-idiotypic reagents are also capable of inhibiting T-cell functions related specifically to the transplantation antigens in question (5-7).

If this clonal diversity of receptors is a property of the precursors of cells reactive to allogeneic transplantation antigens then, theoretically, it should be possible to separate specific precursors from a lymphoid population by adherence to a solid-phase immunoadsorbent bearing the relevant alloantigens. A major objective of such an approach lies in its potential application to clinical organ transplantation and to tumor immunotherapy. In the former case a recipient of a transplant would be prepared for grafting by replacing his immune system with an immunocompetent lymphoid population (perhaps autologous) rendered specifically unresponsive to the intended graft. In the latter situation, a transplanted cytotoxic lymphoid population sensitized to tumor-associated antigens might eliminate a tumor and/or prevent tumor recurrence. This approach would prove useful only if the sensitized cells were depleted of their capacity to generate graft vs. host disease in reaction to the transplantation antigens of the host.

Some previous studies have suggested that immunoadsorption could potentially render normal lymphoid cells tolerant of specific transplantation alloantigens (8–12) although others were unable to cause specific or consistent depletion of reactivity in this way (13–15). Because of this uncertainty regarding a principle with important clinical applicability, we have sought to reinvestigate the question using new and efficient methods of generation of lymphocytes cytotoxic to normal mouse spleen cells which permit immunoadsorption and sensitization experiments to be carried out entirely in vitro. By these means we have been able to demonstrate efficient and highly specific adsorption of committed cytotoxic effector cells, but have been unable to prevent generation of these effectors by exposure of normal or immune lymphocytes

to the same immunoadsorbent before sensitization. To the contrary, we have found a consistent nonspecific augmentation of the capacity of the population to be sensitized after contact with the immunoadsorbent.

Materials and Methods

Animals. Male mice, ages 8–16 wk, were obtained from The Jackson Laboratory, Bar Harbor, Maine. Strain C57BL/10Sn (B10) and the *H-2*-congenic resistant strains on the B10 background, B10.A/SgSn, B10.D2/nSn, and B10.BR/SgSn were used. These will also be designated in subsequent text by the haplotypes of origin of their *H-2K* and *H-2D* regions, *bb*, *kd*, *dd*, and *kk*, respectively.

Media. Eagle's minimal essential medium (MEM) no. 2 (NIH Media Unit, Bethesda, Md.) with penicillin/streptomycin, 25 mM HEPES, and 10% heat-inactivated fetal calf serum was used for cell preparation and cytotoxic assays. Cells for in vitro sensitization were cultured in Eagle's MEM (Microbiological Associates, Bethesda, Md.) with added sodium pyruvate, Eagle's nonessential amino acids, glutamine, penicillin/streptomycin, 5×10^{-5} M 2-mercaptoethanol, and 10% fetal calf serum (lot no. 87517; Microbiological Associates).

Cell Suspensions. Spleens were removed aseptically and teased to form single cell suspensions, filtered through nylon mesh, and pelleted by centrifugation. Erythrocytes were removed by treatment for 1 min with ammonium chloride buffered with potassium bicarbonate followed by washing in four volumes of medium. Viabilities determined by dye exclusion were generally 90–95%.

In Vitro Sensitization. 4×10^6 responders plus 5×10^5 irradiated stimulators (2,000 R) in a 2 ml volume of culture medium were placed in wells of 24-well tissue culture plates (FB16-24TC; Linbro Scientific, New Haven, Conn.). Plates were incubated for 5 days at 37°C in a humidified atmosphere of 3–5% CO_2 in air. Cultures were harvested by repeated aspiration of the wells with a Pasteur pipette. Cultures with 4×10^6 responders and graded numbers of stimulators from 5 to 20 $\times 10^5$ had similar yields of viable cells and varied up to 35% in content of lytic units.

Monolayer Preparation. Immunoadsorbents were spleen cell monolayers prepared by a modification of the methods of Bonavida and Kedar (11) and Stulting and Berke (16). 60-mm Petri dishes (Falcon 3002; Falcon Plastics, Div. of BioQuest, Oxnard, Calif.) were coated with high molecular weight poly-L-lysine (Sigma Chemical Co., St. Louis, Mo.), 50 μg/ml in phosphate-buffered saline, pH 7.2, followed by extensive washing with phosphate-buffered saline. Spleen cells to be converted into monolayers were washed three times with serum-free medium and plated in 2 ml at 25×10^6/ml. Plates were incubated at 37°C for 45 min and then centrifuged at 75–100 *g*, 23°C, for 5 min. The plates were swirled gently for 10 s, the supernates containing nonadherent cells were aspirated, and the plates were washed two to four times by addition of 2 ml of medium with 10% fetal calf serum, gentle swirling, and aspiration of the supernate. Further washes resulted in release of fewer than 10^6 cells per wash. $10–20 \times 10^6$ cells were recovered from each plate and therefore $30–40 \times 10^6$ were adherent. The monolayers were macroscopically and microscopically nearly but not completely confluent.

Adsorption. 20×10^6 cells in 1.5 ml of medium with fetal calf serum were plated on a monolayer, incubated for 45 min at 37°C, and centrifuged at 75–100 *g* at 23°C for 5 min. Nonadherent cells were obtained by swirling gently for 10 s and aspirating the supernate. Adherent cells were eluted by further incubation of the plate at 37°C with 2 ml of 5 mM disodium EDTA in phosphate-buffered saline, pH 6.9, for 15 min with rocking at 10 cycles/min. Cells were recovered by swirling for 10 s, removing the supernate, and washing once in five volumes of medium.

Assay for Cellular Cytotoxicity. Normal spleen cells were labeled with $Na_2{}^{51}CrO_4$ (NEZ-030; New England Nuclear, Boston, Mass.) before use as targets for cytotoxic effector cells. 0.5 or 1.0×10^5 targets in 0.1 ml were plated in 96-well, U-bottom Microtiter plates (IS-MRC-96; Linbro Scientific). Effector cells were adjusted to a standard concentration of viable cells and were plated in 0.1 ml in triplicate for at least three serial twofold dilutions. Plates were centrifuged at 10 *g*, 23°C, for 2 min, and incubated for 3–4 h at 37°C in humidified 5% CO_2 in air. Plates were then centrifuged at 800 *g*, 4°C, for 10 min, and 0.1 ml of the supernate of each well was removed and counted in a Nuclear-Chicago gamma counter (Nuclear-Chicago Corp., Des Plaines, Ill.).

Calculations. Maximum release was taken as total incorporated ^{51}Cr less background. Sponta-

neous release was assessed from targets incubated with medium alone. Specific release caused by effector cells was calculated as [(counts − spontaneous release)/(maximum − background)]. Attacker-type targets were included as controls and specific release of chromium from these targets never significantly exceeded 0% and usually was somewhat less than 0% because of a protective effect of increased cell density. In order to compare activity of unabsorbed cytotoxic populations with the activity of the same population after adsorption to or elution from various monolayers a linear-logarithmic plot of percent lysis (y) vs. attacker to target ratio (x) was fitted with a straight line for each cytotoxic population. Concentration of lytic units was then calculated by the method of Brunner et al. (17) for each population and these values were compared. This method of comparison is valid when the lines do not differ significantly from being parallel and when the range of y's for one line overlaps the range of y's for the other. In many adsorptions of cytotoxic cells and in all adsorptions on a syngeneic responder-type monolayer, these conditions were met but in adsorptions involving the most dramatic reductions in activity, neither condition was met presumably since few cytotoxic cells remained and since those which had not adhered were of low affinity. In these cases, since both populations were always tested over the same range of x's, the depletion was taken to be greater than the ratio of the highest to the lowest x. This method of comparison was conservative in being a minimum estimate of the depletion, but it was inaccurate both in underestimating the loss of cytotoxic units and in failing to reflect the apparent lowering of affinity.

H-2 Typing by Indirect Immunofluorescence. Typing by indirect immunofluorescence was performed as previously described (18). Serum 1483, (B10.A × A)F_1 anti-B10 had a cytotoxic titer against B10 spleen cells of 1:256 and was used at 1:8. Serum 1261, B10 anti-B10.BR, had a cytotoxic titer against B10.A of 1:32 and was used at 1:4. Reactions were developed with a fluoresceinated goat anti-mouse γ_2 rendered specific for the Fc portion by absorption with Fab (kindly provided by Dr. Richard Asofsky, Laboratory of Microbial Immunity, NIAID, NIH, Bethesda, Md.). This reagent bound to less than 2% of splenic lymphocytes and could, therefore, be used to detect lymphocytes coated with exogenous mouse antibodies. Medium controls included goat anti-mouse γ_2 but medium was used instead of immune mouse serum.

Results

Behavior of Cytotoxic Effector Cells. The efficacy of mouse spleen cell monolayers as immunoadsorbents was first examined using cytotoxic cells generated after 5 days of culture. Fig. 1 shows a 17.5-fold or >94% reduction in activity of B10 (*bb*) anti-B10.BR (*kk*) cytotoxic cells after adsorption on the target-type (B10.BR) monolayer, but only a twofold reduction after adsorption on the third-party B10.D2 (*dd*) monolayer. The adsorption on the B10.D2 monolayer may have been partly nonspecific and may have reflected adsorption of activity directed at shared public antigens. In the first 14 consecutive experiments involving various strain combinations, specific adsorption was always seen and the reduction ranged from 88 to greater than 98% with a median of 94%. In seven of these experiments utilizing responder-type monolayers as specificity controls, nonspecific reduction ranged from −4 to 53% with a median of 31%.

Specificity of adsorption was further demonstrated by testing the ability of a monolayer to remove a set of cytotoxic cells with one specificity from a population while sparing a set with another specificity. B10 (*bb*) anti-B10.D2 (*dd*) cytotoxic cells were absorbed on a B10.A (*kd*) monolayer. Fig. 2 A shows a greater than 94% reduction of cytotoxic activity against antigens determined by the *D* end of *H-2* when cells adsorbed on a B10.A monolayer were tested on B10.A targets. The substantial retention of reactivity against B10.D2 targets implies that *K*-end reactivity was spared while *D*-end reactivity was almost totally depleted. The reciprocal experiment involving B10 (*bb*) anti-B10.BR (*kk*) cells adsorbed on a B10.A (*kd*) monolayer showed a greater than 94% reduction of anti-*H-2K*-end activity and retention of *H-2D*-end activity (Fig. 2 B).

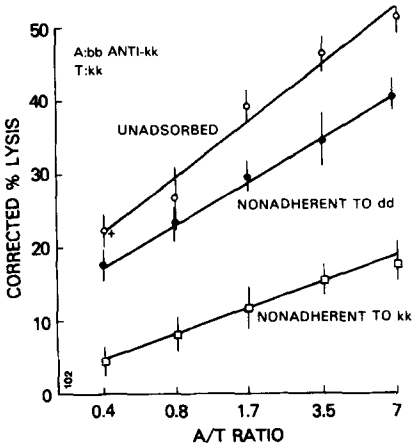

FIG. 1. Adsorption of cytotoxic effector cells on spleen cell monolayers. In this and in subsequent figures, except where noted, abscissa is attacker-to-target (A/T) ratio; vertical lines are SEM; +, spontaneous release; A, attacker; and T, target.

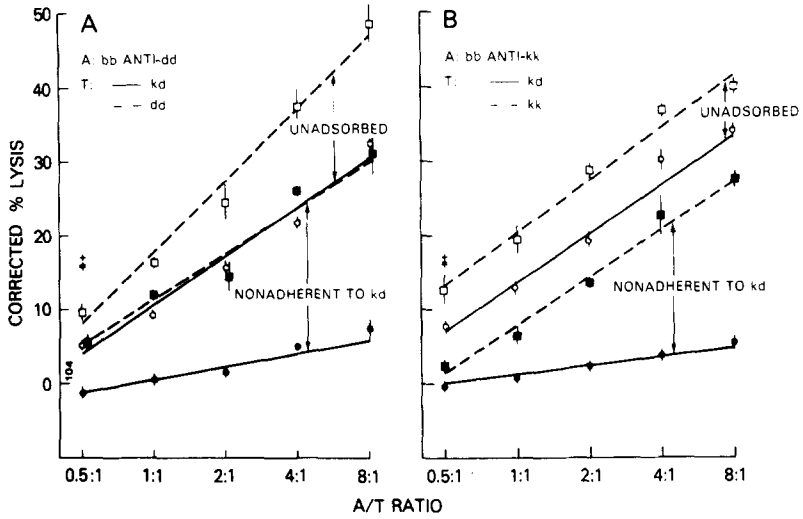

FIG. 2. Separation of cytotoxic subpopulations by adsorption on recombinant monolayers. Spontaneous release: (A) (±), *dd*, (+), *kd*; (B) (±), *kk*; (+), *kd*. A/T, attacker-to-target.

Enumeration of Detached Monolayer Cells in the Nonadherent Fraction of Cytotoxic Populations. Monolayer cells which become detached during an adsorption would function as cold targets in the cytotoxic assay and if present in sufficient numbers could inhibit competitively the lysis of ^{51}Cr-labeled targets. In a preliminary experiment, the number of cells washed free from monolayers incubated with medium alone was found to be fewer than 10^6. This would represent at most 10% of the apparently nonadherent cells from an adsorbed population. However, cytotoxic cells could be expected to damage the monolayer, and therefore, during an actual adsorption, more monolayer cells might be detached. This was investigated by typing a cytotoxic population for H-2 alloantigens after adsorption (Table I). Unadsorbed B10 (*bb*) anti-B10.A (*kd*)

TABLE I

H-2 Typing by Indirect Immunofluorescence of Nonadherent B10 Anti-B10.A after Adsorption on a B10.A Monolayer

	% *kd*	% *bb*
I. Unadsorbed	6*	84
II. Nonadherent	21	73
III. Eluted adherent	25	60

* Medium controls subtracted: group I, 4; group II, 5; and group III, 1.

cytotoxic cells showed a low level of *kk* antigens which reflected adsorbed antigen (19), surviving irradiated B10.A stimulators, or was nonspecific. The nonadherent fraction, which was reduced 87% in cytotoxic activity, showed a 15% increase in cells typing for *kk* antigens and a concomitant decrease in cells typing for *bb*. Cells eluted from the monolayer with EDTA showed a further increase in fraction of cells typing for *kk* and this correlated with the macroscopically visible disturbance in confluence of the monolayer after elution. It was next necessary to determine what fraction of contaminating monolayer cells would give dilutional and competitive inhibition. Cytotoxic cells were mixed with graded numbers of unlabeled targets and then assayed for cytotoxic activity (Fig. 3). A constant number of attackers was used, but the abscissa shows the ratio of attackers plus inhibitors to targets, the apparent A/T ratio, since that is the ratio actually used in adsorption experiments. Populations with less than 20% contamination were not inhibited and only when the contaminating cells numbered more than the cytotoxic cells did inhibition reach the magnitude routinely seen in adsorptions.

Behavior of Precursors. The same adsorptive process was next applied to freshly explanted normal spleen cells just before sensitization in vitro. Despite the highly efficient and specific removal by spleen cell monolayers of cytotoxic effector cells, separation of precursors reactive to specific transplantation antigens was not accomplished. Table II shows the results in two experiments of adsorbing B10 (*bb*) on either a B10 or a B10.A (*kd*) monolayer before in vitro sensitization with B10.A stimulators. Cells nonadherent to the B10.A monolayer became more actively cytotoxic to B10.A targets than did unadsorbed cells. If one assumes that all the cells adherent to the monolayer and all the cells dying during sensitization were irrelevant cells which would have made no contribution to the cytotoxic capacity of the population, then the result of adsorption and sensitization would have been nonspecific enrichment of activity against B10.A. The data in Table II were recalculated (shown in parentheses) as activity per standard number of cells before adsorption in order to test if such nonspecific enrichment could have masked a specific reduction. Since the corrected relative activities of the adsorbed cells were not less than the unadsorbed, there was no evidence for a component of specific adsorption. In one additional experiment enhancement of activity in cells adsorbed on B10.A was seen and in a second additional experiment adsorption had no significant effect. Adsorption on a syngeneic B10 monolayer had a less consistent effect, as in the first experiment (Table II) a nonspecific decrease in activity resulted and in the second experiment an increase resulted. All lysis was specific since killing of control (B10) targets was never significantly greater than 0.

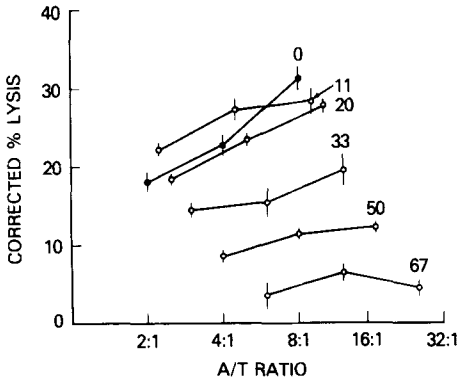

FIG. 3. Dilutional and competitive inhibition of cytotoxicity with cold targets. Effectors are C57BL/10 anti-B10.BR; hot and cold targets are B10.BR. Attacker-to-target (A/T) ratio is (attacker + inhibitor) to target ratio based on addition to a constant number of attackers of a sufficient number of cold targets to give the percent contamination shown by the figure next to each line.

TABLE II

Adsorption of Normal B10 Spleen before In Vitro Sensitization to B10.A

Exp.	Adsorbent	% Recovery		Activity on Target*	
		After adsorption	After culture	B10.A	B10
1	None	–	54	100 (54)	0‡
	B10	75	42	20 (6)	0
	B10.A	58	52	498 (150)	0
2	None	–	74	100 (74)	0
	B10	50	79	255 (101)	0
	B10.A	60	68	169 (69)	0

* Relative activities calculated as described in the Materials and Methods with activity of harvested unadsorbed cells assigned an arbitrary value of 100. The figures in parentheses correct the relative activity for cell loss during adsorption and culture to that of a constant number of normal cells before adsorption.

‡ Activity against B10 targets was assigned the value 0 since no lysis over spontaneous release was seen.

This failure effectively to deplete normal cells of reactivity to specific transplantation antigens might imply (*a*) that some precursors of cytotoxic effector cells lack antigen-specific receptors, (*b*) that they lack receptors at some stage of the cell cycle, or (*c*) that they possess these receptors in such numbers or physicochemical configuration that binding is very weak. Since in vitro sensitization results in a shift from poorly binding precursors to efficiently binding effector cells it would seem possible that sensitization also results in a shift of the binding capacity of precursors. It therefore seemed reasonable to evaluate the secondary response of a population primarily sensitized in vitro and depleted of its cytotoxic cells by adsorption.

Fig. 4 shows B10.D2 (*dd*) anti-B10.BR (*kk*) from an 8-day sensitization culture tested after fractionation on a stimulator-type B10.BR monolayer. A

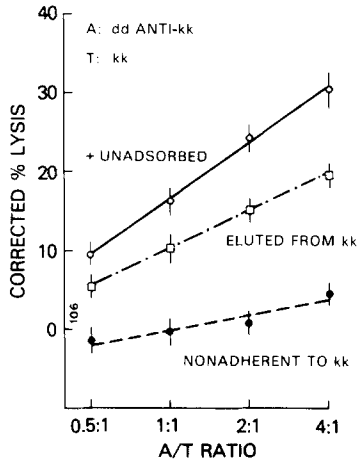

FIG. 4. Adsorption of in vitro primed spleen cells, after 8 days culture. A/T, attacker-to-target.

greater than 88% reduction in activity was seen in the nonadherent fraction. The eluted fraction was less active than the unadsorbed fraction; this was a consistent observation and was probably a consequence of substantial dilutional and competitive inhibition of the eluted population by detached monolayer cells. Both fractions were cultured for a further 3 days with fresh B10.BR stimulators. Surprisingly, the nonadherent fraction was not only undiminished in cytotoxic activity but was in fact about 100-fold more active than the unadsorbed cells (Fig. 5). Similar effects were seen after 2 and 4 days of secondary culture, and unadsorbed cells did not show a peak of comparable magnitude at any of these times. These observations were repeated in four other experiments.

The highly active eluted cells showed a broadening of reactivity since they killed B10 (*bb*) targets to a significant extent, but specificity of killing was seen in the much greater magnitude of lysis of the stimulator-type targets, B10.BR (*kk*). Such populations with augmented activity showed no killing of responder-type targets. The eluted cells were somewhat more active than the nonadherent cells, and this might suggest a segregation of precursors into the adherent fraction. Even so, any segregation was a minor effect in comparison to the augmented activity relative to the unadsorbed cells.

The effect of adsorption on a responder-type monolayer was tested in three experiments and augmentation of cytotoxic activity similar to that seen after adsorption on a stimulator-type monolayer was found (data not shown). Thus the major effect of the monolayer in augmenting responsiveness to boosting in vitro was nonspecific.

If all precursors capable of being stimulated in a secondary culture are progeny of cytotoxic cells and must pass through a cytotoxic phase during primary sensitization, then the optimal time for adsorption of these precursors might be at the peak of the primary cytotoxic response. In our hands peak activity occurs at 5 days and by 8 days cytotoxicity, though still present, is clearly waning, even when culture nutrients are replenished. Therefore the secondary response of cultures adsorbed at 5 days and boosted in vitro was

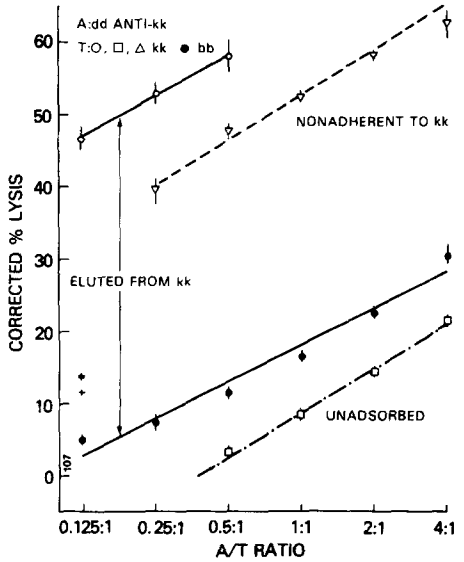

FIG. 5. 3-day secondary sensitization of spleen cells adsorbed on monolayers after 8 days of primary sensitization. (+), *kk* spontaneous release. (±), *bb* spontaneous release. A/T, attacker-to-target.

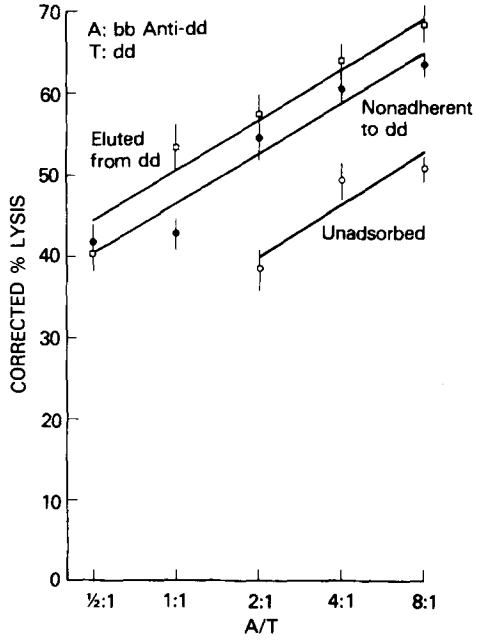

FIG. 6. Secondary sensitization of cytotoxic spleen cells adsorbed at the peak of the primary cytotoxic response. A/T, attacker-to-target.

measured (Fig. 6). Again, cytotoxic populations depleted of activity (94% in this case) were more active after 37 h in culture than the unadsorbed cells. In another experiment the effect was seen as early as 16 h after initiation of the secondary culture. If this resulted from differential stimuli caused by differen-

tial killing of the fresh stimulators by the cytotoxic responder-attackers, then the least cytotoxic population before secondary sensitization should become the most cytotoxic population afterwards. This explanation is unlikely because the cells eluted from the monolayer were more active than the nonadherent cells before secondary sensitization and remained so afterwards.

These results show that in a population primarily sensitized in vitro, precursors of the effector cells resulting from an in vitro boost are present and do not bind efficiently to a monolayer bearing the histocompatibility antigens to which they are reactive, even when a 20-fold reduction of cytotoxic cells in the same population is accomplished. This is true when adsorption is performed at the peak of the primary cytotoxic response or when it is waning.

Discussion

Monolayers formed of normal mouse spleen cells attached to polystyrene coated with poly-L-lysine may be used as immunoadsorbents for the separation of cytotoxic effector cells from mouse spleen cells sensitized in vitro to transplantation alloantigens. We have demonstrated in a quantitative fashion that great efficiency of adsorption can be achieved since 90–95% of the cytotoxic activity of a population can be removed routinely by a single adsorption on the monolayer of appropriate *H-2* type. In quantifying the adsorption, it is important to assess killing at multiple attacker-to-target ratios because comparison of lysis at a single ratio may be misleading. If the slope of the dose response is high, an impressive decrease in lysis at any one attacker-to-target ratio may reflect only a small decrease in activity. Previous studies in other systems have also shown specific adsorption of cytotoxic cells (9, 16, 20–24).

Excess unlabeled targets are capable of competing with labeled targets for cytotoxic effectors and of causing inhibition and apparent depletion. Our studies and those of others (25) indicated a release from monolayers incubated with medium alone of enough monolayer cells to account for no more than 10% of a nonadherent population after adsorption. Assessment of the inhibitory capacity of this fraction of detached monolayer cells revealed very significant inhibition by a fraction of 30% although none by a fraction of 10%. Since the margin between significant inhibition and no inhibition was small and since, under conditions of adsorption when cytotoxic cells come in contact with the monolayer, more extensive detachment of monolayer cells may occur, it is important to assess the detachment directly for any particular conditions of adsorption. Therefore, in the present study we typed nonadherent populations for H-2 alloantigens using indirect immunofluorescence. We found that contaminating monolayer cells were present in greater numbers than estimated, although still in numbers too low to account for any significant proportion of the depletion of activity seen after adsorption (assuming that cells detached from poly-L-lysine-coated polystyrene possess the same inhibitory capacity on a per cell basis as fresh cells). It is impossible to estimate how important such inhibition may have been in previous studies which included no direct assessment of detachment.

Although it is readily possible to prepare a lymphoid population greatly depleted of its cytotoxic activity, we were unable to demonstrate that monolayers could remove sufficient numbers of precursors of cytotoxic effector cells from a normal population to prevent effective sensitization in vitro. Neither were the

monolayers capable of efficiently binding precursors in populations previously primed in vitro even when adsorption removed the residual cytotoxic activity from such populations.

This result differs from several previous studies (8–11), but each of the following problems arises with one or more of these studies. (*a*) Quantification of depletion was lacking either by titration of effectors for calculation on a per cell basis or by total viable spleen cell counts for calculation on a per spleen basis. (*b*) Adsorption may merely have altered the homing properties of the antigen-reactive cells during subsequent in vivo sensitization. (*c*) Specificity of depletion was not shown since retention of capacity to be sensitized to third-party antigens was not tested. (*d*) Cross-reactivity of effectors on different targets was considerable. (*e*) Consistency of the phenomenon was not shown, a point of particular interest in view of Rubin's demonstration of specific or non-specific depletion or enhancement in many different adsorptions (15). Thus, none of these papers demonstrated unequivocally that precursors of cytotoxic cells may be specifically and efficiently adsorbed on monolayers. In addition, failure to adsorb precursors has also been previously reported (13–15).

Our interpretation must be qualified by the formal possibility that adsorption altered in a dramatic fashion the dose response pattern of a fixed number of responders to increasing numbers of stimulators. We observed no evidence to support this possibility and the response of normal spleen cells was relatively insensitive to two- to fourfold increases in stimulator number over the standard number.

In approaching the problem of elimination of the capacity to react to specific transplantation antigens, it is of both conceptual and practical importance to consider the various subpopulations of cells which may participate in the in vitro generation of cytotoxic lymphocytes. The precursor is the antigen-reactive T cell among whose progeny are the cytotoxic effector cells; it is present in peripheral blood, lymph node, and spleen (26–29), and is Ly 2+,3+ (30). The amplifier cooperates with limiting numbers of the precursor to generate a cytotoxic population more active than the summed activities of the two cooperating populations cultured separately; it is present in thymus, and assuming it is essential for generation of cytotoxicity, in lymph node, and spleen (26–29); it is Ly 1+ (30). The presuppressor is an adherent cell whose existence may be inferred from the augmented cytotoxic activity, observed in this study and in others, in populations absorbed on monolayers before sensitization (12, 14, 15). This cell may be related to the adherent T cell of Hodes and Hathcock which matures under culture conditions to generate a potent suppressor (31). Removal of this potent suppressor may account for the augmented activity seen in the present study in adsorbed lymphocytes secondarily sensitized in vitro. This augmented activity was not seen by Kamat and Henney (32) possibly because of the use in their study of cells immunized in vivo and assayed 14 days after primary sensitization. The cytotoxic cell is a theta-bearing, Ly 2+,3+ lymphocyte capable of killing targets bearing appropriate products of the major histocompatibility complex (17, 30). Under prolonged culture the cytotoxic effector cell appears to revert to a small lymphocyte (33), apparently a prekiller which may regain its cytotoxic capacity within 24 h after exposure to antigen (34).

Populations enriched for precursors and poor in amplifiers have been tested

for specific adherence to monolayers; adsorption resulted in a reduction in capacity to be sensitized but the consistency and specificity of this effect have not been fully evaluated (35). Populations rich in amplifier activity or in prekillers have also been tested and were not adsorbable (15, 29, 35). Since in our studies neither normal nor primed spleen cells were adsorbable, it may be inferred that none of the subpopulations, precursors, amplifiers, or prekillers, was adsorbed. An alternative remains that the precursor is present in great excess. Then, in the presence of nonadsorbable amplifiers, even a 90% reduction of precursors may be nullified by synergistic effects of the precursor-amplifier interaction. The postulated suppressor and presuppressor are nonspecific in their effects and are adsorbed nonspecifically.

Only the cytotoxic cell is efficiently and specifically adsorbable. This property of cytotoxic cells strongly implies quantitative or qualitative differences in the receptors of these cells and the receptors of precursors. One possibility is that some precursors at some stages of the cell cycle do not express receptors. Another is that their receptors are few in number or labile so that insufficient binding energy for effective attachment of the cell can be generated. A third possibility is that cytotoxic cells, in furtherance of their physiologic function to attach to and damage antigen-bearing targets, acquire nonspecific attachment mechanisms the activation of which is antigen dependent.

The ability to render an immunologically competent lymphoid population unresponsive to specific transplantation antigens has many investigational and clinical applications. One way to do this may be to expose antigen-reactive cells to a monolayer bearing the antigens in question and to recover the nonadherent cells. Our data thus far indicate this approach is not efficient and suggest that other means of procuring tolerance, as through the use of specific suppressor cells or anti-receptor antibodies must be sought. By contrast, specific adsorption of cytotoxic cells can be performed with such efficiency as to permit dissection of the component specificities in a cytotoxic population.

Summary

Monolayers formed of normal mouse spleen cells attached to polystyrene coated with poly-L-lysine were tested for their ability to bind specifically antigen-reactive cells in normal or primed mouse spleen. 88 to greater than 98% of the activity of cytotoxic populations was removed by a single adsorption. However, normal spleen cells or spleen cells previously primed in vitro could not be depleted of their capacity to be sensitized, even when adsorption effectively removed all residual cytotoxic activity from the same previously primed population. In fact, exposure to an immunoadsorbent augmented the ultimate cytotoxicity generated in a nonspecific fashion. This augmentation was especially dramatic in the case of a previously primed population and may have reflected the removal of a nonspecific suppressor. If antigen-reactive precursors cannot be removed efficiently by adsorption, other approaches to the generation of tolerant lymphoid populations, such as specific suppression of precursor differentiation must be sought.

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