

DIFFERENTIATION OF MEMORY T CELLS TO VIRUS PLAQUE-FORMING CELLS AND CYTOTOXIC T LYMPHOCYTES

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The virus plaque assay has been developed as a potential method for enumerating immunologically activated lymphocytes. It is based on the observation that, while resting lymphocytes are nonpermissive for a variety of RNA viruses, lymphocytes activated by antigens or mitogens are capable of producing virus. When a population of activated lymphocytes is infected with vesicular stomatitis virus (VSV)¹ and plated in agar above a monolayer of L cells, infectious centers or plaques are produced in the indicator layer by individual virus-producing lymphocytes (1).

A variety of evidence has accrued which indicates that this assay is selective for activated T relative to activated B lymphocytes in the mouse (2, 3). In human peripheral blood mononuclear cells, increases in virus plaque-forming cells (V-PFC) are found after stimulation with T-cells mitogens, e.g., phytohemagglutinin (PHA) or concanavalin A (Con A), but not with a B-cell mitogen, *Staphylococcus aureus*, of the Cowan I strain (3, 4).

In earlier studies on development of V-PFC in peripheral blood lymphocytes of tuberculin-sensitive human donors stimulated by purified protein derivative (PPD), it was found that the development of V-PFC was not inhibited by agents blocking mitosis and DNA synthesis (5). From these studies it was concluded that at least some V-PFC were antigen-sensitive, nondividing cells, and it was suggested that there might be an effector cell population in delayed type hypersensitivity with similar properties. Subsequent studies on the development of V-PFC in primary mixed lymphocyte cultures (MLC), however, indicated that the development of V-PFC was totally inhibited by mitotic inhibitors. Further, the V-PFC detected in the I⁰ MLC primarily responded to determinants coded for by I region genes, and not by those coding for H-2D or K end products which might have been expected of cytotoxic T lymphocytes (6). Anti-Ia sera directed against stimulating cells totally blocked development of V-PFC, while the same sera directed against determinants on the responding cells had no more effect than normal mouse sera in inhibiting V-PFC (7).

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¹ Abbreviations used in this paper: Ara C, cytosine arabinoside; Con A, Concanavalin A; CTL, cytotoxic T-lymphocyte; FCS, fetal calf serum; MEM, Eagle's minimal essential medium; I⁰ MLC, primary mixed lymphocyte culture; II⁰ MLC, secondary mixed lymphocyte culture; PBS, phosphate-buffered saline; PFC, plaque-forming cell; PHA, phytohemagglutinin; PPD, purified protein derivative; VPA, virus plaque assay; V-PFC, virus plaque-forming cell; VSV, vesicular stomatitis virus.

The introduction of isoimmune sera which can distinguish functional T-cell subpopulations has made it experimentally possible to distinguish between T cells responsible for helper activity, suppressor activity, MLC reactivity, and H-2 cytotoxic activity.

The purposes of the present experiments were to define the T-cell subpopulation(s) detected by the virus plaque assay and to resolve the question whether cell proliferation was required for development of effector function and virus formation by these subpopulations. It was of special interest to ascertain whether cytotoxic T cells (CTL) could be enumerated by the virus plaque assay.

Materials and Methods

Animals. 2-mo old mice of the C57Bl/6, C57Bl/10 (H-2^b), DBA/2 (H-2^d), and congenic C57Bl/10.D2 (B10.D₂) strains were purchased from The Jackson Laboratory, Bar Harbor, Maine.

Immunization. C57Bl (H-2^b) mice were immunized against H-2^d alloantigens by intraperitoneal injection of 10⁷ P815 mastocytoma cells maintained in DBA/2 mice. For studies on secondary responses of these primed animals, spleen cells were obtained 14–80 days after immunization.

Mixed Lymphocyte Cultures. In each experiment spleen cell suspensions were prepared from normal and primed mice. Single cell suspensions were obtained by teasing the spleens in fetal calf serum and pressing them through a 60-mesh stainless screen into Eagle's minimal essential medium (MEM) containing penicillin (100 U/ml), streptomycin (100 µg/ml), and glutamine (200 mM). Cells were washed, resuspended in MEM supplemented with 20% FCS to a density of 5 × 10⁷ viable cells/ml. Responding cells in the MLC were generally filtered through nylon wool columns for enrichment of T cells by the method of Julius et al. (8). The nonadherent cells, after two washes, were resuspended in complete RPMI 1640 medium (Grand Island Biological Co., Grand Island, N. Y.) containing 5% fetal calf serum (Flow Laboratories, Inc., Rockville, Md.), penicillin and streptomycin as above. In addition 2-mercaptoethanol was added at 5 × 10⁻⁵ M.

Stimulator cells or whole spleen cells were treated with mitomycin C (50 µg/ml, Sigma Chemical Co., St. Louis, Mo.) for 30 min at 37°C, at a cell density of 10⁷ viable cells/ml. For mixed lymphocyte cultures, 5 × 10⁶ stimulator and 5 × 10⁶ responder cells were mixed in 2 ml of 5% fetal calf serum (FCS)-RPMI 1640 and cultured in 16-mm wells of Linbro culture trays (Linbro Chemical Co., New Haven, Conn.) (catalogue no. FB 16-24TC) for 24 or 48 h in most experiments, at 37°C in a humidified atmosphere containing 7% CO₂ in air. For some cultures, cytosine arabinoside (Ara C, catalogue no. 2135, Sigma Chemical Co.) was added to appropriate samples at a final concentration of 10 µg/ml at the initiation of culture.

For in vitro sensitization of T cells, the method of Cerottini et al. (9) was used. Normal C57Bl spleen cells (25 × 10⁶) were admixed with an equal number of mitomycin C-treated DBA/2 or B10.D2 stimulator cells in 30-ml culture flasks. The cells were collected after 13 days in culture, their viability determined by trypan blue exclusion and they were stimulated in secondary MLC as described above.

Controls in all experiments included responding cells cultured alone, generally by using 2.5 × 10⁶ primed or unprimed C57Bl spleen T cells added to an equal number of the same cells, mitomycin C-treated.

Isolation of Ly Subclasses. Anti-Ly 1.2 and anti-Ly 2.2 + Ly 3.2 sera generously provided for these studies were prepared by Dr. E. A. Boyse, Memorial Sloan Kettering Cancer Center, and absorbed and calibrated by Dr. Harvey Cantor, Department of Medicine, Harvard Medical School. Methods for their preparation have been previously described in detail (10–12).

For isolation of Ly subclasses, 50 × 10⁶/ml nylon wool-purified responder cells were incubated with Ly antiserum at a final dilution of 1/40–1/80 in phosphate-buffered saline (PBS) containing 10% FCS for 30 min at room temperature. After washing once, the cells were suspended in 1 ml of selected rabbit serum as a source of complement diluted 1:12 in PBS and incubated for another 30 min at 37°C (13).

Determination of Cell Proliferation. At the appropriate time after culture in I⁰ or II⁰ MLC, 2 × 10⁵ viable cells were transferred into microtiter plates in 0.2 ml of 5% FCS-RPMI 1640 and pulsed for 6 h with 2 µCi of [³H]thymidine (New England Nuclear, Boston, Mass., sp act 2 Ci/mM). Cells were harvested in a Titertek multiple cell culture harvester (Skatron A/S, Flow Laboratories,

Inc., Rockville, Md.) and radioactivity was counted in a Beckman LS-230 liquid scintillation counter (Beckman Instruments, Inc., Cedar Grove, N.J.) at 33% efficiency.

Assay for CTL. The assay for CTL was based on that described by Cerottini and Brunner (14). Because of the complexity of the experimental procedures being carried out on the responding cells, in most experiments cytotoxicity was assayed at 16 rather than 6 h, although in a smaller number of experiments 6-h data were obtained and the pattern of response found to be identical. P815 mastocytoma cells, maintained by serial passage in DBA/2 mice were labeled with 100 μ Ci of 51 Cr and washed and 5×10^4 labeled cells were added to 2×10^6 viable lymphocytes obtained from the MLC in 12×75 -mm culture tubes containing 1 ml 5% FCS-RPMI 1640 (Grand Island Biological Co.). The tubes were incubated at 37°C in an atmosphere of 7% CO₂. At the end of the incubation period, generally 16 h, the cells were centrifuged at 900 *g* for 10 min, and 0.5 ml of supernates were transferred with an Eppendorf pipette into new tubes and counted in an LKB gamma counter. (LKB Instruments, Inc., Rockville, Md.) Specific 51 Cr release was calculated by the method of Cerottini and Brunner by using spontaneous leakage and freeze-thaw controls (14).

Virus Plaque Assay. The virus plaque assay was performed according to the method described by Kano et al., and Jimenez and Bloom (2, 15). Cells were obtained from the MLC at various times (2×10^6 viable cells), washed and infected with VSV at a multiplicity of infection of 50 PFU/cell. The number of specifically activated V-PFC (Δ V-PFC) was calculated as follows: Δ V-PFC/ 10^3 = V-PFC/ 10^3 stimulated cells - V-PFC/ 10^3 nonstimulated cells.

Results

Relationship between Cell Proliferation, Cytotoxicity, and Generation of V-PFC. The principal model for study in these experiments has been a II⁰ MLC reaction since it generates considerably more CTL than primary reactions (9). A comparison of the kinetics of thymidine incorporation, T-cell cytotoxicity, and the formation of V-PFC in I⁰ and II⁰ MLC (Fig. 1) indicates that previous immunization accelerates the tempo of reactivity and increases the peak reactivity of cytotoxic T cells and V-PFC, with little change in the development of proliferating activity. Introduction of Ara C, a potent inhibitor of cellular DNA synthesis, into the primary MLC resulted in the total inhibition of development of CTL and V-PFC during the first 24 h. These activities remained at those levels for at least 48 h, although greater numbers of both were found after 48 h in cultures not treated with Ara C. Identical results (data not shown) were obtained from lymphocytes previously stimulated *in vitro* in a I⁰ MLC and later re-exposed to allogeneic cells in a II⁰ MLC. These experiments indicated that in II⁰ MLC development of V-PFC and CTL paralleled one another, and these functions appeared to be carried out both by dividing cells and by cells capable of differentiation without cell division.

Effect of VSV Preinfection on Development of Cytotoxic T Cells. We have previously found that preinfection of resting lymphocytes with VSV later followed by stimulation by mitogens or allogeneic cells resulted in a dramatic inhibition of thymidine incorporation by the preinfected cells. We have interpreted these results to indicate that resting lymphocytes are capable of being infected by lytic viruses, but that the infection remains latent or persistent until the cells are activated (3, 16). Since VSV is a lytic virus, stimulation of the preinfected cells resulting in activation of the virus is a lethal event for the cell. Because of the parallelism in the kinetics of CTL and V-PFC, the possibility of eliminating these cells by preinfecting them with virus was suggested. Accordingly, immune spleen cells were infected overnight with VSV at a multiplicity of infection of 25. After three washings, the cells were restimulated by allogeneic cells in the presence of Ara C. As shown in Fig. 2, preinfection resulted in an almost total elimi-

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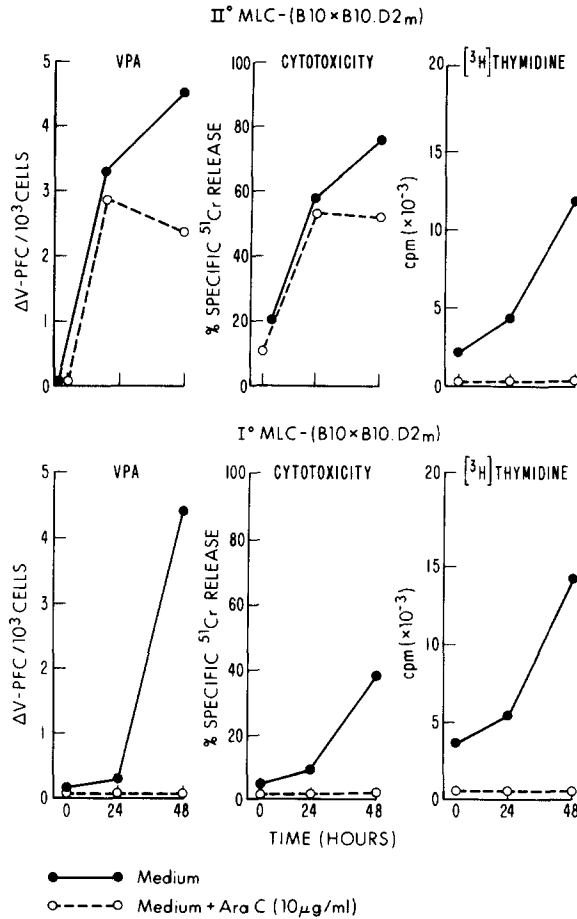


FIG. 1. Lymphocyte activation in primary and secondary MLC as detected by the VPA, ^{51}Cr -release and incorporation of $[^3\text{H}]$ thymidine. Responder cells in secondary MLC were primed 21 days before MLC. Solid lines indicate response in culture medium alone; dotted line in medium containing Ara C $10\ \mu\text{g}/\text{ml}$.

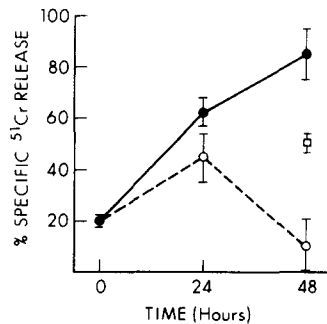


FIG. 2. Effect of preinfection of lymphocytes with VSV on development of CTL in secondary MLC. Lymphocytes obtained 24 days after sensitization were infected with VSV for 24 h or cultured in medium alone before stimulation with allogeneic cells. Solid line, noninfected responder cells; dotted line, VSV preinfected responder cells; box, noninfected responding cells cultured with Ara C.

TABLE I
 Comparison between Unfractionated Immune Spleen Cells and T-Cell Subpopulations
 in II^o MLC (21 Days after Primary Immunization)

Cells	Treatment	Killed	Δ V-PFC/ 10 ⁶	Specific ⁵¹ Cr release	Incorporation of [³ H]thymi- dine	V-PFC	Cytotox- icity	[³ H]Thymi- dine incor- poration
		%				%	%	%
(A) Unfractionated	NMS	18.7	6.84	56.5	21,900	100	100	100
(B) Ly 1	anti-Ly 2	26.5	4.95	12.0	15,100	72	21	70
(C) Ly 2, 3	anti-Ly 1	36.4	2.20	88.4	4,600	32	156	21
(B + C) Ly 1 + Ly 2, 3			6.61	50.6	14,890	98	90	68

nation of cytotoxic activity 48 h after stimulation whereas Ara C resulted in only a 50% diminution. These data directly indicate that VSV can infect and inactivate those T cells capable of differentiating upon contact with antigen into cytotoxic effector cells.

Virus Production by Ly 1 and Ly 2,3 Cells. From the studies of Shiku et al. (11) and Cantor and Boyse and Cantor and Weissman (10, 12, 17) it would be expected that T cells capable of generating cytotoxic activity in secondary MLC would express the Ly 2,3 phenotype, while T cells responsible for the majority of [³H]thymidine incorporation would have the Ly 1 phenotype.

If our interpretation of the previous experiments is correct, then V-PFC should be contained in activated T cells of both the Ly 1 and Ly 2,3 populations. Table I illustrates the responses of spleen T cells from donors primed 21 days earlier with allogeneic cells after treatment with anti-Ly 1 and anti-Ly 2,3 sera. Ly 1 cells were responsible for 70% of V-PFC, while Ly 2,3 cells accounted for 30% of V-PFC. These relative proportions were found to be remarkably constant among six experiments carried out by using primed spleen cells, treated with the Ly isoantisera and boosted in vitro over a wide range of times after primary immunization (see below, and Fig. 4). Reconstitution of cells treated with anti-Ly 1 and cells treated with anti-Ly 2 serum produced a population deficient in Ly 1,2,3 cells, and this Ly 1 + Ly 2,3 mixture contained the same level of virus plaque-forming activity as found in untreated cells. Since the precise number of Ly 1,2,3 cells in the population is not known, it is not possible formally to exclude the possibility that some V-PFC were of this phenotype, although their contribution could not be great.

Discrimination between Memory T Cells and Prekiller T Cells. The data in Table I indicate that the stimulated Ly 2,3 cells incorporated threefold less [³H]thymidine than comparable numbers of Ly 1 cells. The question of most interest to us, however, was whether Ly 2,3 memory cells for cytotoxicity required proliferation to differentiate into functional killer T cells after restimulation with alloantigens. To clarify this question, the differing patterns of virus plaque-forming activity of the T-cell subclasses were studied in the presence and absence of Ara C (Fig. 3). It emerged that unfractionated spleen cells obtained approximately 3 wk after immunization, and restimulated 24–48 h in the presence of Ara C generated approximately the same number of V-PFC as the Ly 2,3 population alone or in the presence of Ara C. These results demonstrate that the primed Ly 2,3 population can be activated by alloantigens to produce virus and cytotoxicity in the absence of proliferation. This result further suggested the

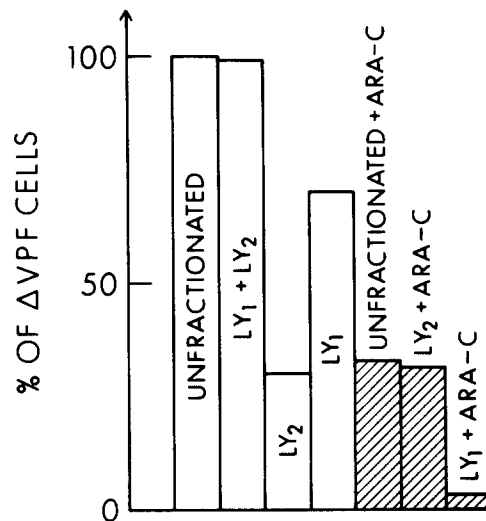


FIG. 3. Contribution of T-lymphocyte subpopulations to V-PFC (21 days).

possibility that if this were an invariant function of Ly 2,3 memory cells, then their number could be estimated without the necessity of anti-Ly 1 treatment simply by addition of Ara C to the cultures. However, when similar experiments were carried out by using spleen cells from donors primed for longer periods of time (Fig. 4), it was found that an increasing proportion of Ly 2,3 differentiation to V-PFC and cytotoxic effector cells was dependent on cell proliferation. At 80 days after priming, for example, essentially all of the Ly 2,3 cells capable of being activated could be inhibited by addition of Ara C, as measured both in the virus plaque assay in the cytotoxic test. In both assays there was a complete agreement between the results of NMS plus Ara C treatment and anti-Ly 1 plus Ara C treatment indicating that the proliferation at later times was truly a property of the Ly 2,3 cells, and independent of effects mediated by the Ly 1 cell. In this situation (data not shown), there was a marked increase in [³H]thymidine incorporation by the stimulated Ly 2,3 cells, essentially comparable to that given by the Ly 1 cells at this time.

These results indicate that the longer after immunization, the greater the number of Ly 2,3 cells requiring a proliferative stage before acquiring the capability of producing virus or carrying out cell-mediated cytotoxicity. In this regard, these cells behave as true memory cells, requiring both additional antigen stimulation and cell proliferation to differentiate fully into competent effector cells.

Discussion

These experiments were designed to clarify two general questions: (a) the functional nature and subpopulation characteristics of the T cells detected by the virus plaque assay; and (b) whether cell proliferation was required for acquisition of effector function and ability to replicate virus by these subpopulations. We have chosen II⁰ MLC as the system for study both because high levels of T-cell proliferation and cytotoxicity are generated (9, 18, 19) and because the subpopulations carrying out these functions have been defined by anti-Ly isoanti-

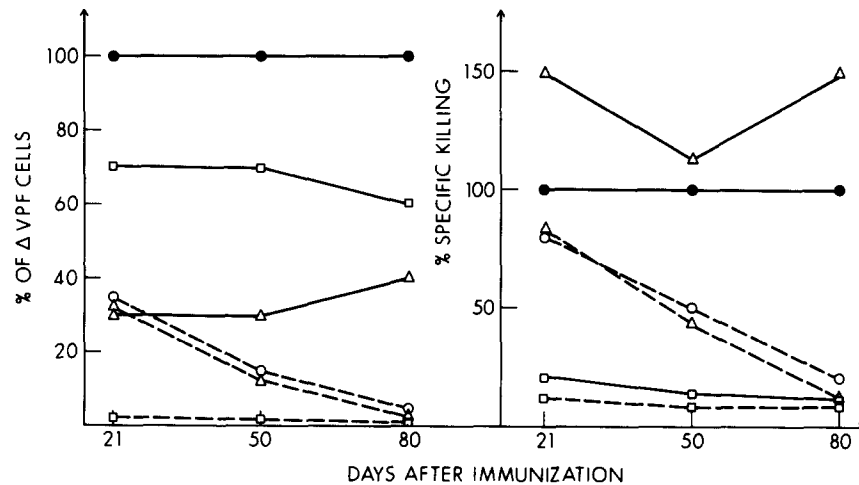


FIG. 4. Differentiation of memory cells obtained at various intervals after immunization into V-PFC and CTL. ○—○, unfractionated spleen lymphocyte preparation; □—□, Ly 1 cells. △—△, Ly 2,3 cells. Solid line, cells cultured in medium alone; dotted line, cells cultured in medium containing Ara C.

sera. Cantor and Boyse have demonstrated in the same MLC combination as used here that the proliferating T cell has the Ly 1 phenotype and the CTL has the Ly 2,3 phenotype, and that both functional subpopulations derive from an Ly 1,2,3 precursor cell (17, 19).

In previous studies on murine spleen cells, marked stimulation in V-PFC was produced by T-cell mitogens such as PHA, Con A, or pokeweed mitogen; much smaller or negligible responses were produced by the B-cell mitogen lipopolysaccharide. In I^0 MLC the V-PFC was found to be completely eliminated by treatment, either before or after culture, with anti-thy 1 serum plus complement, formally demonstrating that it was a T cell (2).

In the present experiments an accelerated course of development of V-PFC has been observed in secondary MLC as compared with primary MLC, as has been found to be the case in assays for incorporation of thymidine and T-cell cytotoxicity. When spleens primed *in vivo* or *in vitro* were subjected to treatment with anti-Ly 1 or anti-Ly 2,3 sera plus complement, a relatively constant percentage of V-PFC was eliminated (Table I, Figs. 3 and 4). Anti-Ly 1 + C treatment reduced the number of V-PFC above background by approximately 70%, and treatment with anti-Ly 2,3 serum plus complement reduced the number of V-PFC by approximately 30%. While reconstitution experiments failed to indicate that activated Ly 1,2,3 cells are detected by the virus plaque assay that possibility cannot be formally excluded.

We believe that the present data provide three lines of evidence to support the contention that the virus plaque assay, in addition to detecting the proliferating Ly 1-type T cell, is capable of detecting cytotoxic T lymphocytes. First, in the presence of Ara C in the II^0 MLC, there is a clear parallel in the development of cytotoxic T lymphocytes and V-PFC in the total absence of [3 H]thymidine incorporating cells (Fig. 1). Secondly, treatment with anti-Ly 2,3 serum + C eliminates 30% of the V-PFC and essentially 100% of the cytotoxic activity, without significantly diminishing incorporation of [3 H]thymidine (Table I, Fig. 3).

Lastly, in previous studies it has been reported that resting lymphocytes can be infected by VSV, and this infection remains latent or persistent until the cells are activated (3, 16). In the present experiments, preinfection of responding primed spleen cells with VSV for 24 h, followed by stimulation *in vitro* resulted in the total elimination of cytotoxic T cells at 48 h, under conditions in which simply inhibition of cell proliferation, with Ara C, would have resulted in a diminution of only 50%. These results provide direct evidence that the cytotoxic T lymphocyte is detected by the virus plaque assay.

The level of study in the present experiments has been primarily a qualitative one, and optimal conditions for quantitative detection of the actual number of V-PFC or cytotoxic T lymphocytes have not yet been established. With indirect estimations of the number of CTL in different experimental circumstances, different laboratories have obtained often widely divergent estimates. For example, Fitch et al. (18) in repeated *in vitro* boosting experiments estimate maximally 33% of surviving lymphocytes must be CTL. In limiting dilution studies Bach et al. (20) have estimated the number of CTL in a II⁰ MLC to be 0.1–1%, and most recently Thorn and Henney (21) have provided an estimate of approximately 0.8–2%. The data in the virus plaque assay are comparable to the latter figures, although this figure is a minimum estimate since it is unlikely that the VPA is detecting all activated T cells of any class.

The second major concern of this work has been to resolve the apparent discrepancies in different systems on whether cell division is required for differentiation of memory cells into effector cells in cell-mediated immunity. Previous studies on development of V-PFC *in vitro* after stimulation of tuberculin-sensitive human lymphocytes by PPD had indicated that the development of V-PFC was not inhibited by the mitotic inhibitors, vinblastine or Ara C (5). These experiments suggested the existence of an antigen-sensitive nondividing cell, and it was proposed that such cells would be short lived and have effector function in cell-mediated immune reactions. While this point has been controversial, it has subsequently been reported that not only V-PFC, but T-cell helper activity (22), migration inhibitory factor production (23, 24), and T-cell cytotoxicity (25–27) may be generated in secondary responses from memory cells in the absence of cell proliferation.

In the present experiments comparison of the effects of Ara C on the development of I⁰ and II⁰ MLC confirmed that in primary MLC all *in vitro* functions measured in the primary response, namely incorporation of [³H]thymidine, development of CTL, and V-PFC were abolished by the inhibitor of DNA synthesis. In contrast, in II⁰ MLC by using spleen cells from recently primed animals, there was essentially no diminution either in V-PFC or CTL in the first 48 h of culture (Fig. 4). These observations suggested the possibility that it might be possible to estimate the number of CTL in other species for which anti-Ly-like isoantisera are not available simply by carrying out the VPA in the presence of inhibitors of DNA synthesis. However, systematic study showed that the development of both CTL and V-PFC responses of Ly 2,3 cells became progressively more susceptible to Ara C as the time interval between primary immunization and *in vitro* boosting was increased (Fig. 4).

Thus, there appears to be a marked difference in the immediate precursor to the CTL and V-PFC depending on time after contact with antigen. One possible

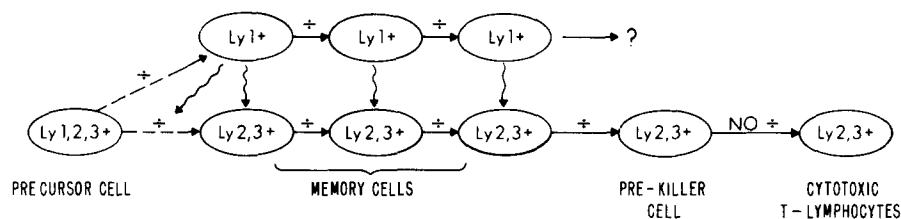


FIG. 5. Model for differentiation of CTL. Both Ly 1 and Ly 2,3 cells derive from a common precursor which is Ly 1,2,3 positive (17). The inhibition of all functions in I^0 MLC by Ara C indicates that the early differentiation process requires cell proliferation. Subsequent to antigen stimulation, Ly 2,3 memory cells arise which are defined by their ability upon further contact with specific antigen to differentiate into functional CTL after one or more cell divisions. In the scheme, the amplifying effect of the Ly 1 proliferating T cells, or a product derived from them (wavy lines) is viewed as being most important during the initial stages of differentiation. The present data would suggest that there is an Ly 2,3 cell, incapable of carrying out effector function without further antigen stimulation, but capable of differentiating into a CTL or V-PFC without division. We have termed this antigen-sensitive, nondividing cell a prekiller cell.² Since pre-killer cells are not found in spleens late after immunization, it may be inferred that they are short-lived cells.

model for visualizing this process of T-cell differentiation is illustrated in Fig. 5, and is analogous to models for differentiation of B cells proposed by Askonas and Williamson (28).

The discrepancy between the number of actual killer cells at a given time, particularly late after immunization, and the number of CTL capable of being generated by further exposure of memory cells to antigen raises the question of which of these two parameters is more likely to be a useful index of the effector function in clinical states. For example, if this scheme applied to the specific effector cells operative against tumor antigens, it is conceivable that at any given time in some patients there might be very few directly measurable cytotoxic T lymphocytes, but a rather large pool of memory cells capable of differentiating into cytotoxic lymphocytes. In other patients, there might be a negligible pool of memory cells. For this reason, we suggest that it may be important to attempt to measure not only the actual cytotoxic circulating cells in such individuals, but also their cytotoxic potential, i.e., the number of cytotoxic cells capable of being generated upon appropriate antigen stimulation *in vitro*.

Summary

The aims of this study were to define the T-cell subpopulation(s) detected by the virus plaque assay, and particularly to determine whether the virus plaque assay could be used to enumerate cytotoxic T lymphocytes. In addition, studies were undertaken to ascertain whether cell proliferation was required for development of cytotoxic effector function and virus plaque formation by these subpopulations. The results of experiments with a secondary mouse mixed lymphocyte culture (MLC) model indicated that 70% of virus plaque-forming cells bore the Ly 1 phenotype and 30% the Ly 2,3 phenotype. Three lines of evidence sug-

² The term prekiller cell was originally used by Kamat and Henney (29) to designate a primed cell which acquired cytotoxic activity in the absence of antigen stimulation by culturing in medium alone. Such cells have been consistently observed in the present work as well but are distinguished here from cells differentiating to CTL without division upon antigen stimulation.

gested that cytotoxic T lymphocytes (CTL) can be detected by this assay: the fact that some virus plaque-forming cells (V-PFC) bear the same Ly phenotype as CTL; the use of an inhibitor of DNA synthesis indicated that proliferating cells could be eliminated with no effect on V-PFC production and cytotoxic activity of the Ly 2,3 cell population; and that infection of primed lymphocytes with vesicular stomatitis virus before (MLC) stimulation eliminated cytotoxic activity.

In primary MLC, development of V-PFC and CTL was completely abolished by cytosine arabinoside. In contrast, in secondary MLC, some CTL and V-PFC were generated by antigenic stimulation in the absence of proliferation. However, the development of both functions became progressively more susceptible to cytosine arabinoside as the time between primary immunization and in vitro boosting is increased. It is suggested that there may be a considerable disparity between the number of existing effector cells at any given time and the cytotoxic potential, i.e. the number of cells capable of being generated by antigenic stimulation.

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