CYTOTOXIC T-CELL ACTIVATION
BY POLYRIBONUCLEOTIDES: DNA SYNTHESIS
IS NOT REQUIRED*

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The activation requirements of T lymphocytes, especially cytotoxic T lymphocytes, has been a matter of considerable controversy. Cytotoxic effector T cells have been shown to be specifically induced after in vivo or in vitro immunization and polyclonally induced by T-cell mitogens (1-3). T-cell cytotoxic capacity is found 5-7 days after alloimmunization or 24-48 h after mitogen activation. These results imply that some reprogramming of the cell is necessary before the expression of effector functions. Activation of cytotoxic T cells has been found to be enhanced by T-T cell collaboration (4-6), to be dependent of the presence of macrophages (7), and to require DNA synthesis and cell division (8-10).

T lymphocytes can be stimulated by complexes of synthetic polyadenylic:polyuridylic acid (poly A:U)[1] causing enhancement of T-cell helper activity (11, 12). Poly A:U enhancement of helper functions cannot be explained by a mitogenic effect of the polynucleotides on T lymphocytes and is probably due to a stimulated production of antigen-nonspecific T-cell factors (13, 14). Such factors also interfere with the induction of tolerance to a T-dependent antigen-soluble bovine gamma globulin (15, 16).

Poly A:U has been shown to enhance reactivity in mixed lymphocyte culture cell-mediated lympholysis and both poly A:U and the related complex of polyinosinic:polycytidylic acid delay tumor onset or growth in several systems (17-19). Since poly A:U's enhancing activity in humoral immune systems occurs early in the response, it is possible that this is the important time interval for enhancement of cell-mediated systems as well. Here we present evidence that poly A:U complexes cause polyclonal activation of cytotoxic T cells, in the absence of antigen, which can be demonstrated within 9-18 h in vitro against radiolabeled allogeneic target cells. The absence of a requirement for DNA synthesis, as well as the short time period necessary for the appearance of cytotoxic activity, suggests that the requirements for cytotoxic cell development may be less involved than was previously thought.

Materials and Methods

Animals. A/sn, A.CA, B10.5M, CBA, (CBA × C57BL/10)F₁, heterozygous Nude/+ on BALB/c background, and normal BALB/c mice were bred and maintained in our animal colony. Athymic

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[1] *Abbreviations used in this paper:* BSS, balanced salt solution; Con A, concanavalin A; LPS, lipopolysaccharide; PBS, phosphate-buffered saline; PHA, phytohemagglutinin; poly A:U, polyadenylic:polyuridylic acid.

Nude BALB/c mice were obtained from Bomholtgård, Ry, Denmark. Animals were age- and sex-matched for each experiment.

Spleen Cells. Spleens were aseptically removed and cell suspensions prepared by means of teasing with sterile forceps. After brief sedimentation, the cells in the supernatant fluids were washed with balanced salt solution (BSS), resuspended in culture medium, counted, and adjusted to the appropriate concentration.

Macrophage-Depleted Spleen Cells. Macrophage-depleted spleen cells were prepared using the carbonyl iron method of Lundgren et al. (20). Carbonyl iron powder (0.2 g) (General Aniline and Film Corp., New York), sterilized by ultraviolet irradiation, was added to 20 ml of spleen cell suspension (2×10^7 cells/ml) in a 250 ml sterile flask. The mixture was incubated at 37°C for 30 min in a shaking water bath. After incubation, the iron-adherent cells were removed using a strong magnet and the remaining cells were washed three times with BSS, counted, and resuspended at the appropriate concentration in culture medium.

Tumor Cells. Moloney virus-induced leukemia cells YAC (H-2^a) were obtained from the Department of Tumor Biology, Karolinska Institute, Stockholm, Sweden and were passaged in ascites form in A/sn mice. After removal from the peritoneal cavity, YAC cells were washed in BSS and resuspended in culture medium for labeling as described by Forman and Möller (21). Briefly, YAC cells (10^6/ml) were cultured in the presence of 0.05–0.1 μCi/ml ^{125}I-5-iodo-2-deoxyuridine in a sterile flask at 37°C for 6 h on a rocking platform. Labeled cells were washed with BSS and resuspended at 10^6 cells/ml in culture medium. Routinely, 10^5 labeled cells contained 3,500–6,000 cpm.

Culture Medium. Mishell-Dutton culture medium was prepared from components purchased from Flow Laboratories, Irvine, Scotland and was supplemented with HEPES buffer (0.01 M; Flow Laboratories). All media contained 10% heat-inactivated fetal calf serum (Reheis lot N50003; Armour Pharmaceutical Co., Phoenix, Ariz.) unless otherwise noted.

Polynucleotides. Synthetic homopolymers of adenylic and uridylic acid were purchased from Miles Laboratories, Slough, England (lots 83,85 and 90,93,96, respectively). The homopolymers were dissolved in phosphate-buffered saline (PBS), pH 7.2, at 5 mg/ml. Complexes of poly A:U were prepared by mixing equal volumes of the individual homopolymer stock solutions at room temperature for 10 min. Poly A:U was then diluted in PBS to the desired concentration.

Mitogens. Concanavalin A (Con A) was purchased from Pharmacia Fine Chemicals Ltd., Uppsala, Sweden. Lipopolysaccharide (LPS) from *Escherichia coli* O55:B5 was obtained from Dr. T. Holme, Department of Bacteriology, Karolinska Institute, Stockholm, Sweden. Mitogens were dissolved in serum-free culture medium before use.

Drugs and Chemicals. Mitomycin C (Sigma Chemical Co., St. Louis, Mo.) was dissolved in sterile water and diluted in serum-free culture medium before use. Silica was a gift of Dr. B. Andersson, Department of Tumor Biology, Karolinska Institute, Stockholm, Sweden. Silica particles were suspended in BSS and heat sterilized before addition as fine suspensions to culture.

Radioisotopes. ^{125}I-5-iodo-2-deoxyuridine (3.91 μCi/mmol) and ^{3}H-methyl-thymidine (5 μCi/mmol) were purchased from The Radiochemical Centre Amersham, England. Radioisotopes were diluted in serum-free medium for addition to culture.

Cytotoxic Assay. To determine the cytotoxic response, 10^7 effector cells were cultured in 3-cm Petri dishes with 10^5 labeled target cells (optimal ratio) in a 1.0 ml vol for 15–18 h at 37°C in a rocking, gas-tight chamber containing 7% O_2, 10% CO_2, and 83% N_2 unless otherwise stated. After incubation, effector and target cells were resuspended using a rubber policeman, transferred to hemolysis tubes, centrifuged, and the supernatant fluids collected (Sup 1). Each cell pellet was resuspended in 0.3 ml of 0.25% trypsin for approximately 1 min to disrupt dead cells that had failed to lyse. 0.7 ml of BSS was then added to each tube to stop the reaction. The tubes were centrifuged as before and one-half the supernatant fluids collected (Sup 2). Tubes containing Sup 1, Sup 2, or the cell pellet were counted in a Nuclear Chicago gamma-spectrometer (Nuclear-Chicago Corp., Des Plaines, Ill.) and the percent isotope release calculated according to the following formula:

$$\% \text{ Isotope release} = (\text{Sup } 1 + 2 \text{ Sup } 2 - 3 \text{ Bg})/(\text{Sup } 1 + \text{Sup } 2 + \text{Pellet} - 3 \text{ Bg}),$$

where Bg = machine background. In this system, background release (from control cultures) was in the range of 15–40%. Triplicate samples were assayed for each experimental group.

Assay of DNA Synthesis. Spleen cells (5×10^5) were cultured for 48 h in microplates (NUNC, Roskilde, Denmark) in 0.15 ml of culture medium containing the desired stimulating substance.
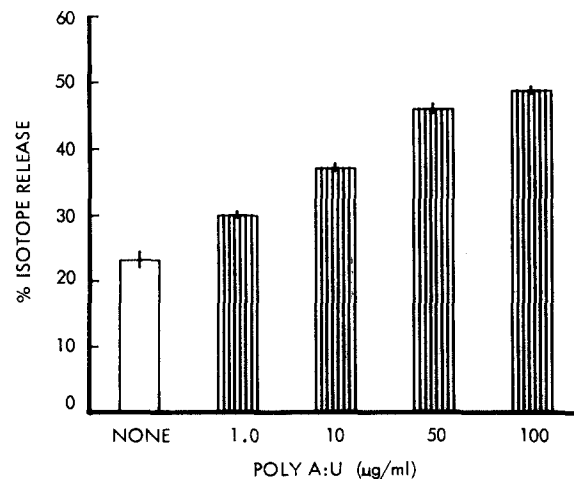
FIG. 1.   Cytotoxic cell dose response to poly A:U. 10 million spleen cells were cultured with 10⁵ YAC target cells in the presence (striped bars) or absence (open bar) of various concentrations of poly A:U. Cytotoxicity was assayed 18 h later. The mean response ± standard error of triplicate samples is presented for each group.

18 h before harvest, [³H]thymidine (1.0 µCi/0.02 ml/well) was added. Cultures were harvested onto glass fiber filters using a microharvester (Flow Laboratories), the filters were dried, and counted using a Packard liquid scintillation counter (Packard Instruments, Inc., Downers Grove, Ill.). The mean and standard error was calculated for data from triplicate samples.

Results

Activation of Cytotoxic Spleen Cells by Poly A:U.   To determine whether poly A:U could nonspecifically activate spleen cells to express cytotoxic effector functions, varying doses of poly A:U were added to 10^7 (CBA × C57BL/10)F$_1$ spleen cells with 10⁵ radiolabeled YAC target cells and cultured for 18 h. As seen in Fig. 1, an increase in cytotoxic capacity accompanied increasing poly A:U concentration up to 100 µg/ml. In other experiments poly A:U doses from 0.0001 to 0.1 µg/ml were found to slightly suppress the response while 500 µg/ml caused no greater activation than did 100 µg/ml. As a control for direct poly A:U toxicity, 10⁶ YAC target cells were cultured alone or with 100 µg/ml poly A:U in the standard culture system and assayed for isotope release after 18 h. Poly A:U was never observed to cause target cell death (see Fig. 2). Therefore, the observed cytotoxic effects were the result of poly A:U's stimulatory effect on the spleen cell population.

Kinetics of Poly A:U-Induced Cytotoxicity.   Cytotoxic effector lymphocytes have been shown to be induced after 5–6 days in culture with allogeneic cells or after 1–2 days culture with Con A. Since the induction period of humoral immune responses can be shortened after poly A:U stimulation, it was of interest to determine the optimal time period for the induction and expression of cytotoxicity after poly A:U activation. Accordingly, 10^7 (CBA × C57BL/10)F$_1$ spleen cells were incubated with 100 µg/ml poly A:U and 10⁵ labeled YAC target cells under standard culture conditions and cytotoxicity was measured at 3-h intervals from 6 to 24 h. As seen in Fig. 2, significant cytotoxicity, above unactivated control cultures, was observed after 12 h and continued to increase
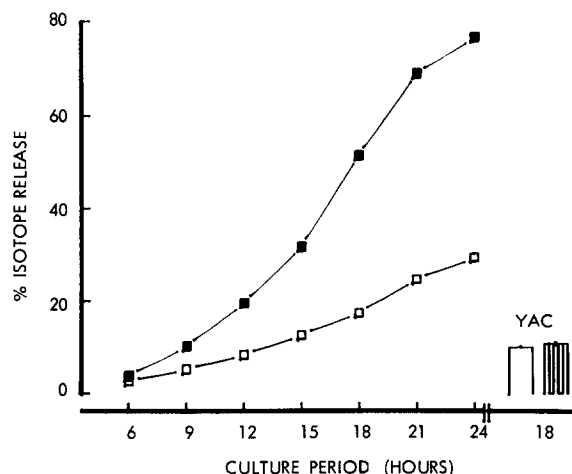
FIG. 2. Kinetics of poly A:U-induced cytotoxicity. 10 million spleen cells were cultured with 10^5 YAC target cells with (filled squares) or without (open squares) 100 μg/ml poly A:U. Cytotoxicity was assayed at 3-h intervals. As a control for poly A:U toxicity, 10^6 YAC target cells were cultured with (striped bar) or without (open bar) 100 μg/ml poly A:U and cytotoxicity was measured after 18 h. The mean response ± standard error of triplicate cultures is presented for each group.

throughout the culture period. These results indicate that all the prerequisite cellular requirements for the induction and expression of cytotoxic cells can occur within 12–24 h after activation. Using cromium-labeled YAC target cells, activation and killing could be demonstrated within 8 h (% isotope release in activated cultures 32.27 ± 0.31, % background isotope release 20.25 ± 0.84). Furthermore, poly A:U is capable of inducing cytotoxicity in a shorter time period than Con A in a similar system (3).

Poly A:U Activation of Cytotoxic Cells is Antigen Independent. Poly A:U has been shown to enhance T-lymphocyte helper functions in the absence of antigen (14). To test the capacity of poly A:U to stimulate cytotoxic cells in a similar fashion, 10^7 (CBA × C57BL/10)F_1 spleen cells were incubated with 100 μg poly A:U/10^7 spleen cells/ml at 37°C in a shaking water bath for varying periods of time. After incubation, the cells were washed four times with BSS, resuspended to the original volume in fresh culture medium, and assayed for cytotoxic capacity. The results, Fig. 3, demonstrate that the spleen cells need only be exposed to poly A:U for 2 h to induce the same degree of cytotoxic potential that occurred when poly A:U was present throughout the culture period. Using ^{3}H-poly A:U, the content of poly A:U remaining in the activated cell population was determined after incubation and washing. Less than 0.04 μg poly A:U/10^7 spleen cells remained after four washes. This dose is well below that necessary to cause activation. Therefore, we conclude that poly A:U activation of cytotoxic spleen cells occurs within 2 h and is antigen independent.

Poly A:U-Induced Cytotoxicity is Dependent upon T Lymphocytes. Since T and B lymphocytes, as well as macrophages, have been implicated as effector cells in various cytotoxic systems, it was important to determine which cell(s) was activated by poly A:U. Similarly, the possible requirement for macrophages needed clarification.
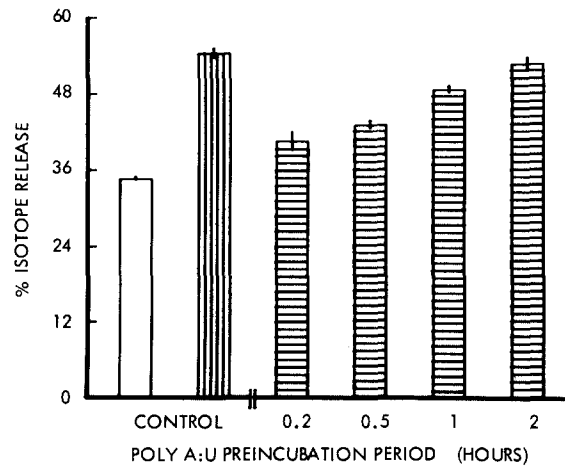
FIG. 3. Activation of cytotoxic cells by short-term incubation with poly A:U. Spleen cells (10^7/ml) were incubated with 100 μg/ml poly A:U for various periods of time (horizontally striped bars). After incubation, the cells were washed in BSS, resuspended to the original volume, and cultured with 10^5 YAC target cells. For comparison, 10^7 spleen cells and 10^5 target cells were cultured with (vertically striped bar) or without (open bar) 100 μg/ml poly A:U throughout the culture period. Cytotoxicity in all groups was assayed after 18 h. The mean response ± standard error of triplicate cultures is presented for each group.

TABLE I

Effect of T-Cell Depletion on Poly A:U-Induced Cytotoxicity

Effector cells*	% Isotope release‡		% Net release§
	Control	Activated	
A			
1 CBA × C57BL/10	15.1 ± 1.0	36.6 ± 2.4	21.5
BALB/c Nude/+	13.6 ± 0.3	37.5 ± 2.0	23.9
BALB/c Nude	39.2 ± 1.0	48.2 ± 0.1	9.0
2 CBA × C57BL/10	33.6 ± 0.6	47.8 ± 0.7	14.2
BALB/c Nude/+	15.5 ± 0.3	31.8 ± 0.4	16.3
BALB/c Nude	18.3 ± 0.4	20.8 ± 0.6	2.5
B			
CBA normal	45.2 ± 0.7	87.1 ± 1.4	42.0
CBA AT × BM	28.1 ± 1.3	42.4 ± 1.2	14.3

* 10 million spleen cells were cultured with (activated) or without (control) 100 μg/ml poly A:U and 10^5 YAC target cells.
‡ Cytotoxicity was measured after 18 h. The mean response ± standard error of triplicate cultures is presented for each group.
§ % Net release = % isotope release activated − % isotope release control.

To assess the necessity of thymus-derived spleen cells for the induction and expression of poly A:U-induced cytotoxicity, spleen cells from BALB/c Nude, BALB/c Nude/+ heterozygous, and (CBA × C57BL/10)F$_1$ mice were cultured with 100 μg/ml poly A:U and 10^5 YAC target cells. Cytotoxicity was measured after 18 h. The results, Table I, show that Nude spleen cells have an impaired cytotoxic potential when compared to Nude/+ heterozygous or normal spleen
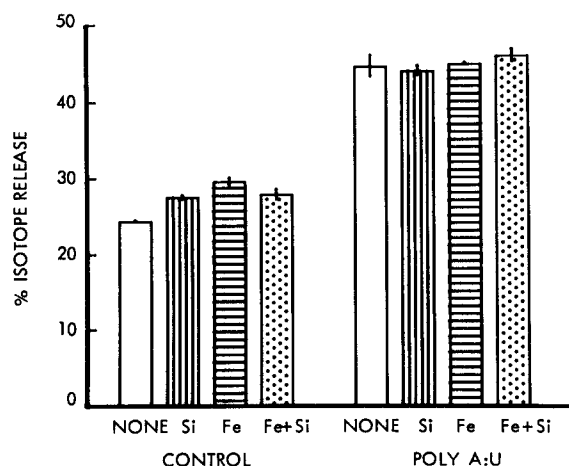
FIG. 4. Effect of macrophage depletion on poly A:U-induced cytotoxicity. 10 million normal (open bars) or iron-treated (Fe, horizontally striped bars) were incubated with or without 100 µg/ml poly A:U for 2 h, washed four times in BSS, counted, and resuspended in fresh culture medium. Subsequently, poly A:U-activated or control cells were cultured with 10^5 YAC target cells in the presence (Si, vertical striped bars) or absence of silica (150 µg/ml). Iron-treated control or poly A:U-activated cells were also cultured in the presence of silica (Fe + Si, stippled bars). Cytotoxicity was measured after 18 h. The mean response ± standard error of triplicate cultures is presented for each group.

cells. Similar results were obtained using spleen cells from adult-thymectomized, irradiated and bone marrow-reconstituted mice (AT × BM) (Table I) although T-cell depletion in these animals was not complete. The mitogenic responsiveness of AT × BM spleen cells to Con A was reduced to 20% of that seen in cultures of normal spleen cells. The LPS response, however, was unaffected. These results allow us to conclude that poly A:U-induced cytotoxicity is T-cell dependent.

In some systems the generation of T-cell-mediated responses requires the presence of macrophages or macrophage culture supernates (7, 22). The macrophage contribution to poly A:U-induced cytotoxicity was determined by removing macrophages using carbonyl iron, silica, or both combined. Macrophages were removed before and/or after spleen cell activation with poly A:U to probe the necessity of macrophages for induction and expression of cytotoxic cells in this system. 10 million normal or iron-treated (CBA × C57BL/10)F$_1$ spleen cells were incubated with or without 100 µg/ml poly A:U for 2 h at 37°C, washed four times, counted, and resuspended to the original concentration. To assess the possible effector function of macrophages, poly A:U-activated or control cells were cultured with or without 150 µg/ml silica and 10^5 YAC target cells in the standard assay system. Silica has been shown to be selectively toxic for cultured macrophages and this dose of silica abolishes the primary in vitro immune response to sheep erythrocytes and prevents the development of mature macrophages from precursor cells (23, 24). The results from one such experiment are presented in Fig. 4. The depletion of macrophages before or after poly A:U activation had no effect upon the observed cytotoxicity. A slight increase in the background cytotoxicity is seen after macrophage depletion.
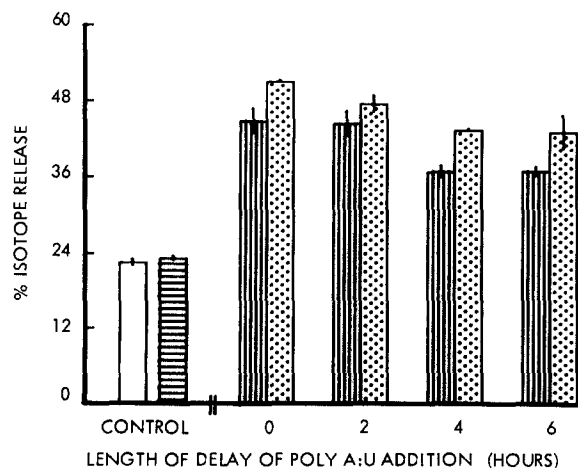
FIG. 5. Effect of silica addition to culture before poly A:U activation. 10 million spleen cells were cultured with (stippled bars) or without (vertical striped bars) silica (150 μg/ml) for 0–6 h before the addition of 100 μg/ml poly A:U. Control cells were cultured with (horizontally striped bars) or without silica (open bar) but did not receive poly A:U. YAC target cells (10^5) were added 6 h after culture initiation and cytotoxicity was assayed 18 h later. The mean response ± standard error of triplicate cultures is presented for each group.

Since macrophage depletion by silica may require a short time period to be complete, spleen cells were exposed to silica for varying periods of time before activation. 10 million spleen cells were cultured with or without 150 μg/ml silica for 0–6 h before the addition of 100 μg/ml poly A:U. YAC target cells (10^5) were added 6 h after the initiation of culture and cytotoxicity was assayed 18 h later. As shown in Fig. 5, normal spleen cells cultured in the absence of silica were capable of being activated when poly A:U was added at the initiation of culture or 6 h later. Addition of silica at 0 time served only to slightly enhance the cytotoxic response at each time period. However, silica had no effect on background cytotoxicity in control cultures indicating that silica was not toxic for the YAC target cells nor could silica activate cytotoxic cells itself. Silica was capable of exerting its macrophage-depleting effect within 2 h as determined by the abolition of mitogenic responsiveness of spleen cells to dextran sulfate. The latter response has been shown to be macrophage dependent (U. Perrson, personal communication).

From these results it can be concluded that splenic T cells are required for the development of poly A:U-induced cytotoxicity. Furthermore, activation and expression of the cytotoxic cell population does not require macrophages.

Specificity of Poly A:U-Activated Cytotoxic Cells.   Since poly A:U has been shown to be capable of inducing cytotoxic cells in the absence of antigen, it became important to determine the specificity of killing by these cells. If poly A:U activation of T cells is polyclonal it would be expected that cytotoxic cells, capable of killing YAC target cells (*H-2ᵃ*), could be induced in all strains allogeneic to the target. To test this hypothesis, 10^7 spleen cells from A/sn (*H-2ᵃ*), A.CA (*H-2ᶠ*), B10.5M (*H-2ᵇ*), CBA (*H-2ᵏ*), or (CBA × C57BL/10)F₁ (*H-2ᵏ/ᵇ*), mice were cultured with 10^5 YAC target cells for 18 h with or without poly A:U and assayed as usual. Spleen cells from all strains allogeneic to the target were
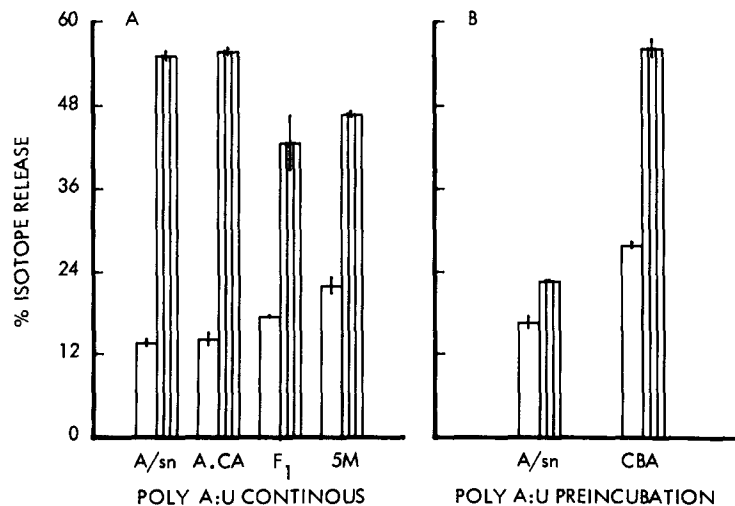
Fig. 6. Effect of short-term poly A:U activation on the ability to kill syngeneic target cells. 10 million spleen cells were cultured with 10^5 YAC target cells in the presence (striped bars) or absence (open bars) of 100 μg/ml poly A:U (A). In another experiment, spleen cells (10^7/ml) were incubated with (striped bars) or without (open bars) 100 μg/ml poly A:U, washed seven times in BSS, resuspended in fresh medium, and cultured with 10^5 YAC target cells (B). In both experiments cytotoxicity was assayed after 18 h. The mean response ± standard error of triplicate cultures is presented for each group.

capable of killing. Surprisingly, syngeneic A/sn spleen cells also demonstrated cytotoxic activity in the presence of poly A:U (Fig. 6 A). This syngeneic killing may be the result of cytotoxic A/sn spleen cells, specific for allogeneic *H-2* haplotypes, causing nonspecific lysis after being brought into contact with the target cell by cell-bound poly A:U. A similar situation has been shown to exist in Con A-induced cytotoxic systems (3, 25). However, if the spleen cells were activated by a 2 h exposure to poly A:U and washed extensively to remove the cell-bound polynucleotides, only cells allogeneic to the target were capable of killing (Figure 6 B). Therefore, poly A:U induces cytotoxic cells capable of killing allogeneic but not syngeneic target cells in the absence of a nonspecific binding ligand. This suggests that the activation of cytotoxic cells by poly A:U is most likely polyclonal.

Activation of Cytotoxic Cells does not Require DNA Synthesis. It has been reported that DNA synthesis is required for the generation of cytotoxic cells by alloantigens or mitogens (8–10). In contrast, there is at least one report of cytotoxic cell activation independent of DNA synthesis (26, 27). To determine whether DNA synthesis accompanied poly A:U activation of cytotoxic cells, normal A/sn or CBA spleen cells were activated by poly A:U (100 μg/ml) during a 2 h incubation at 37°C. After seven washes with BSS, control and activated cells were assayed for cytotoxic and DNA synthetic activity. Cytotoxicity was measured after 18 h while DNA synthesis was assayed after 48 h. As shown in Fig. 7, poly A:U-activated cells did not incorporate [³H]thymidine. However, cultures of activated cells responded to both LPS and Con A, indicating that activated cells were capable of synthesizing DNA and that poly A:U activation did not interfere with incorporation of [³H]thymidine. That cytotoxic cells were
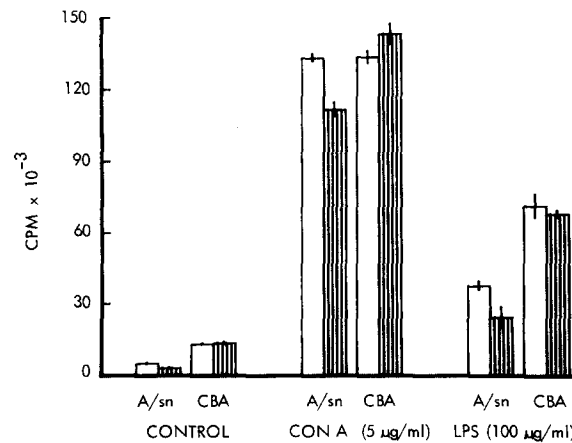
FIG. 7. Effect of poly A:U activation on DNA synthesis. 10 million A/sn or CBA spleen cells were incubated with (striped bars) or without (open bar) 100 μg/ml poly A:U for 2 h. After seven washes in BSS, the cells were resuspended to the original volume and cultured alone or with 5 μg/ml Con A or 100 μg/ml LPS in microcultures (5 × 10^5 cells/well) for 48 h. ^{3}H-thymidine was added 18 h before harvest. The mean response ± standard error of triplicate samples is presented for each group.

indeed activated is reflected in the level of cytotoxicity observed. A/sn spleen cells expressed a net cytotoxicity of 2% (28% activated − 26% control) while allogeneic CBA spleen cells gave a net cytotoxicity of 25% (65% activated − 40% background). Similar results were seen when unlabeled YAC cells were present in the mitogen-stimulated cultures, indicating that target cells did not affect the ability to measure DNA synthesis.

Results from experiments in which mitomycin C was used to block DNA synthesis before activation show that the induction of cytotoxicity was not inhibited while DNA synthetic responses to Con A and LPS were abolished (Table II). In these experiments, B10.5M spleen cells (2 × 10^7/ml) in serum-free media) were incubated with mitomycin C for 45 min at 37°C. After two washes to remove excess mitomycin C, treated or normal spleen cells were cultured with or without 100 μg/ml poly A:U and assayed for cytotoxic activity against 10^5 YAC target cells. As a control for the inhibition of DNA synthesis, mitomycin C-treated or normal spleen cells were activated with Con A or LPS and incorporation of [^{3}H]thymidine was measured after 48 h. Mitomycin C pretreatment did not inhibit net cytotoxic activity but eliminated mitogen responsiveness. Mitomycin C did not affect the release of isotope from YAC targets since background cytotoxicity did not increase; actually it was reduced. Spleen cells, incapable of DNA synthesis, were competent to mount a cytotoxic response after poly A:U activation.

Discussion

We have described a new system in which the activation requirements of cytotoxic cells can be examined over a short time period independent of antigen. Optimal activation of cytotoxic cells occurred after a 2 h exposure to poly A:U and could be demonstrated after a 9–12 h incubation with allogeneic target cells.

TABLE II

Effect of Inhibition of DNA Synthesis on Poly A:U-Induced Cytotoxicity

Mitomycin C*	% Isotope release		% Net release ‡	Con A§		LPS§	
	Control	Activated		cpm‖	% Inhibition¶	cpm‖	% Inhibition¶
μg/ml							
0	34.9 ± 0.2	64.2 ± 0.8	29	110,687	0	92,589	0
15	25.5 ± 0.3	47.0 ± 2.9	22	3,633	97	23	100
20	24.7 ± 0.3	42.7 ± 1.3	20	898	100	69	100
25	26.2 ± 0.7	41.3 ± 0.9	16	262	100	0	100

* Spleen cells (2×10^7/ml) were incubated with mitomycin C in serum-free medium for 45 min at 37°C and washed twice.

‡ % Net release = % isotope release from cultures containing activated cells − % isotope release from cultures containing control cells.

§ 1.0 μg/ml Con A or 100 μg/ml LPS were added to cells in microculture (5×10^5 cells/well).

‖ Background subtracted.

¶ % Inhibition = cpm from cultures of treated cells/cpm from control cultures (no mitomycin C treatment).

Maximal cytotoxicity was seen after 21–24 h in culture. The short time period required for induction and expression of cytotoxicity suggests that few requirements need be met for activation to occur. Indeed, using ^{51}Cr-labeled target cells activation and killing could be observed within 8 h. In contrast, alloantigen activation requires a number of days and is enhanced by cellular cooperation (4–8) while polyclonal stimulation by Con A requires 24 h to develop maximal cytotoxicity (3). Furthermore, activation by alloantigens or Con A has been reported to require DNA synthesis and/or cell division (8–10). Synthesis of DNA is not required for poly A:U-activated cytotoxicity since DNA synthesis does not occur after poly A:U stimulation and inhibition of DNA synthesis does not inhibit the response. Because of the short time interval between activation and assay (15–18 h) in this system, a role for cell cooperation seems unlikely but is not ruled out.

That the cytotoxic effects observed are dependent upon T lymphocytes is supported by the experiments using Nude or AT × BM mice as a source of effector cells. The potential for development of cytotoxicity is lost or dramatically reduced in animals depleted of T cells. The removal of macrophages did not affect, or slightly enhanced, the activation or expression of poly A:U-induced cytotoxicity. This eliminates the macrophage as the effector cell and rules out any helper cell role.

Poly A:U activates effector cells polyclonally since spleen cells allogeneic to the target (non-*H-2ᵃ*) cause target cell lysis after activation while A/sn cells do not. However, if poly A:U is present in the assay culture nonspecific lysis will occur; A/sn spleen cells kill YAC. This is probably due to poly A:U acting as a binding agent between activated killer cells and syngeneic targets. A similar situation exists in lectin-induced cytotoxic systems and is due to nonspecific killing of targets by effector cells lacking clonally distributed recognition receptors (3, 25).

Target and effector cell incompatability at *H-2* is sufficient for poly A:U-induced cytotoxicity to be expressed. Thus, spleen cells from A.CA (*H-2ᶠ*), sharing the background but not the *H-2* genetic component with the YAC target (*H-2ᵃ*), are competent to lyse the target in the absence of any binding ligand (unpublished observation). Whether other antigens contribute to killing in a totally allogeneic situation is not known.

It appears most likely that poly A:U is activating cytotoxic T cells directly and polyclonally. Poly A:U activation is fundamentally different from primary alloantigen or lectin activation in that the induction period is very short and DNA synthesis is not required. These results are in sharp contrast with those of Nedrud et al. and Heininger et al. who state that DNA synthesis is obligatory for cytotoxic cell activation by alloantigens (9) or Con A (10), respectively. However, attempts to demonstrate poly A:U mitogenicity for T cells have been unsuccessful (reference 13, and data reported here). It is possible that poly A:U activates the same T-cell populations activated by alloantigens or Con A but triggering occurs at a later stage in the activation pathway requiring few steps to full activation. Alternatively, poly A:U may activate only relatively mature cells that do not require DNA synthesis or cell division to express cytotoxic functions. Since this induction is polyclonal, a large proportion of T cells should be activated, abrogating the need for clonal expansion required in an antigen-dependent clonally restricted response. A similar situation exists in the secondary CML response where cytotoxic cells are rapidly induced in the absence of DNA synthesis (27).

Poly A:U has been shown to polyclonally activate T cells to produce nonspecific factors capable of enhancing humoral immune responses in vitro as well as blocking the induction of tolerance to soluble bovine gamma globulin in vivo (14, 15). The findings reported here confirm poly A:U's activity as a polyclonal T-cell activator and extend earlier findings to include activation of cell-mediated cytotoxic responses. Furthermore, these observations suggest that the adjuvant activity of poly A:U is due to polyclonal T-cell stimulation. Such polyclonal stimulation is likely to activate cells with suppressive as well as helper activity, the overall response being a result of a balance between help vs. suppression (C. W. Morris and A. G. Johnson, personal communication).

Since poly A:U activates cytotoxic lymphocytes in the absence of DNA synthesis, this system offers the opportunity to study activation independent of nonessential cellular functions accompanying other forms of stimulation. Findings similar to ours have been reported using human peripheral blood lymphocytes and phytohemagglutinin (PHA) in a short-term killing assay against human fibroblast monolayers (26). In that system, killing was nonspecific since PHA was present throughout the assay and the effector cells were not delineated. However, activation and killing was independent of DNA, RNA, and protein synthesis although metabolic activity was required (26). Whether other similarities exist between their results and those reported here cannot be determined.

Using poly A:U's ability to directly activate cytotoxic T cells, one can investigate whether the absence of cytotoxic reactivity in experimentally induced tolerant situations is due to a lack of potential effector cells, active suppression, or the result of a helper cell defect. We are presently investigating such situations.

Summary

We have shown that cytotoxic T cells can be polyclonally activated by a short exposure to complexes of polyadenylic:polyuridylic acid (poly A:U). Activation is optimal at a dose of 100 μg/ml poly A:U and occurs during a 2 h incubation period in the absence of antigen. Killing of allogeneic, but not syngeneic, target cells can be observed after 12 h in culture and peaks after 21–24 h in the absence of any nonspecific binding ligand. The observed cytotoxicity is mediated by T lymphocytes and does not require accessory macrophages or DNA synthesis for the activation or expression of effector functions. These results suggest that few requirements exist for the activation of cytotoxic T cells.

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