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Doxorubicin-induced Alterations in Cultured Myocardial Cells Stimulate Cytolytic T Lymphocyte Responses

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Doxorubicin treatment of cultured murine myocytes induces alterations capable of stimulating $CD8^+$ cytolytic T lymphocyte (CTL) responses in vitro. ***Lymphocytes from normal BALB/cBy female mice were cultured for times varying from 2 to 10 days on syngeneic neonatal myocytes treated with doxorubicin, and surviving lymphocytes were assayed for cytotoxicity to drug-treated and untreated myocyte targets. Maximal cytolytic responses occurred on day 8. Activity to treated targets was twice that observed with untreated myocytes. Concentrations of the drug as low as 10^{-8} mol/l (molar) for as little as 30 minutes initiated the necessary antigenic changes, although treatment with 10^2-fold higher concentration for 4 to 6 hours appeared optimal. Cytotoxicity of the lymphocyte population could be eliminated by pretreatment of the sensitized effector cells with either anti-Thy 1.2 or anti-Lyt 2 monoclonal antibody and complement, while treatment with complement alone or anti-L3T4 monoclonal antibody and complement were ineffective. Myocardial cells were not the only tissue-derived targets susceptible to lysis by the drug-induced CTL. Lymphocytes stimulated on doxorubicin-treated myocytes demonstrated even better cytotoxicity to drug-treated neonatal kidney cells, but showed only minimal activity to neonatal liver or skin fibroblasts. Concentrations of doxorubicin per 10^6 cells was greatest for kidney cells followed by heart and liver cells. Skin fibroblasts incorporated the least amount of the drug. Therefore, there was a partial correlation between sensitivity of drug-treated targets to immune cytolysis and drug uptake. (Am J Pathol 1990, 137:449–456)***

The anthracycline antibiotics, of which doxorubicin is a member, are extensively used in chemotherapy of cancer.[1,2] However, these drugs can have certain well-known and potentially dangerous side effects that limit usefulness once specific dose thresholds are attained.[3,4] Perhaps the best described of these side effects is a cardiomyopathy characterized by vacuolization of the myocytes with accompanying mitochondrial alterations and cell death. Lymphocytic infiltrates are not usually associated with this form of heart disease,[5,6] but have been observed concomitantly with tissue injury in at least one report.[7] The highly toxic effects of the drug on cellular metabolism directly induce myocyte dysfunction, and cytolysis of sufficient numbers of myocardial cells may surpass the ability of the surviving cells to perform normal cardiac functions. Undoubtedly, these toxic effects of the drug are the predominant cause of tissue injury. However, other pathogenic mechanisms might also exist that could aggravate myocardial damage in some individuals. Drug therapy could initiate immune responses to heart cells, leading to indirect myocyte destruction. Autoreactive antibodies and spleen cells have been described in rabbits treated with this drug that appear to have broad tissue specificity.[8] Additionally, doxorubicin modulates immune responses to other antigens, having either enhancing or suppressing activity, depending largely on the time and dose of drug administration.[9–11] These investigations suggest a broad, polyclonal, and possibly nonspecific interaction between doxorubicin and the immune system. A second possibility is that the drug binds to the plasma membrane, and acts as a hapten, resulting in drug-specific immune responses. Monoclonal antibodies have been developed to the drug, indicating that doxorubicin possessed antigenic characteristics for humoral immunity.[12] The present study suggests that the drug triggers cellular responses as well.

Materials and Methods

Animals

BALB/cBy mice were originally obtained from Jackson Laboratory, Bar Harbor, ME. Neonatal and adult female

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animals of this strain were supplied from a breeding colony of these mice maintained at the University of Vermont.

Drug

Doxorubicin hydrochloride (Adriamycin RDF, ADRIA Laboratories, Columbus, OH) in 10-mg single-dose vials contained 10 mg of the drug, 50 mg lactose, and 1 mg methylparaben in powder form. Aliquots of the drug were measured on the day of use, dissolved in saline to 1 mg/ml, and subsequently diluted in Dulbecco's minimal essential medium (DMEM, Grand Island Biological Co. [GIBCO], Grand Island, NY) containing 5% fetal bovine serum (FBS, GIBCO), penicillin, and streptomycin.

Preparation of Primary Cell Cultures

Neonatal mice less than 3 days of age were killed by hypothermia, and dipped in 70% ethanol. Skin, liver, kidneys, and heart were removed aseptically, minced finely, washed free of blood, and subjected to sequential enzymatic digestion with an equal mixture of 0.4% collagenase (Worthington Biochemical Co. Freehold, NJ) and 1% pancreatin (GIBCO) until single cell suspensions were obtained. The cells were repeatedly washed with DMEM-5% FBS, resuspended in fresh DMEM containing 5% FBS and 10% horse serum to a concentration of 1×10^6 cells/ml, and dispensed into tissue culture plates.[13]

Fluorometric Evaluation of Doxorubicin Concentrations in Cultured Cells

Primary cell cultures were obtained as described above, and incubated at 37°C for 90 minutes with 10^{-6} mol/l (molar) doxorubicin in DMEM. The monolayers were washed and removed using a rubber policeman. The cells were dissociated in versene (GIBCO), counted by trypan blue exclusion, and evaluated for drug incorporation using the fluorometric method described by Donelli et al.[14] Briefly, the cells were pelleted, resuspended in 1 ml H_2O, and homogenized. Next, 0.3 ml of a 33% solution of $AgNO_3$ and 0.25 ml H_2O were added to the cells and the mixture was agitated for 20 minutes. Finally, 3 ml butyl alcohol was added to each sample and agitated for 1 minute. The layers were separated by centrifugation at 300*g* for 3 minutes. The doxorubicin that was extracted into the butyl alcohol layer was read on an SLM-4800 Spectrofluorometer (SLM Instruments Inc., Urbana, IL) at 470 nm using a gain of 1.0. Adsorbance values of extracted samples were compared with adsorbance values of standard doxorubicin solutions at 10^{-6}, 10^{-7}, and 10^{-8} mol/l.

Preparation of Lymphocytes

Mesenteric lymph nodes from normal 6- to 8-week-old mice were removed, and the lymphocytes were retrieved by pressing the nodes through a fine mesh screen. The cells were washed in DMEM-5% FBS and viability was determined by trypan blue exclusion.

In Vitro *Generation of Cytolytic T Lymphocytes (CTL)*

The procedure for *in vitro* generation of CTL from normal lymph node cells has been described previously.[15] Briefly, 100 μl of the myocyte suspension was dispensed into each well of 96-well tissue culture plates (Corning Scientific Products, Corning, NY) and incubated for 2 days at 37°C in a 6% CO_2-94% air-humidified NAPCO model 5300 incubator (Portland, OR) to allow cell attachment. The monolayers were washed and overlaid with DMEM-5% FBS alone or containing concentrations of doxorubicin as indicated in the text. The monolayers were again washed 3 times with medium, fixed for 30 seconds with a 0.3% glutaraldehyde solution in Hank's balanced salt solution (HBSS, GIBCO), washed 3 times, and incubated for 2 hours at 37°C in DMEM-5% FBS. At the end of the incubation period, the monolayers were again washed, then overlaid with 1×10^6 lymphocytes in 0.2 ml DMEM-5% FBS containing 5×10^{-5}M 2-mercaptoethanol. The plates were cultured in 6% CO_2 at 37°C for the times indicated. The lymphocytes were retrieved, washed, and resuspended in fresh medium for use in the cell-mediated cytotoxicity assay. Viable cell recoveries ranged from 10% to 35% of the added lymphocyte population.

Cell-mediated Cytotoxicity Assay (CMC)

Approximately 10^3 target cells were cultured for 2 days in 4.7-mm diameter tissue culture wells (Bellco Glass Inc., Vineland, NJ) at 37°C, washed, and overlaid with 10 μl medium containing either 1 μCi ^{51}Cr ($Na_2{}^{51}CrO_4$, NEN, Boston, MA) alone or the radioisotope and virus/drug as indicated in the text. After incubation for 2 hours at 37°C, the monolayers were washed 4 times with medium and overlaid with 10 μl DMEM-5% FBS containing *in vitro* sensitized lymphocytes or nonimmune lymph node cells from normal animals. Control cultures consisted of wells with medium but no lymphocytes. The assays were incubated for 18 hours at 37°C at an effector:target cell ratio of 100:

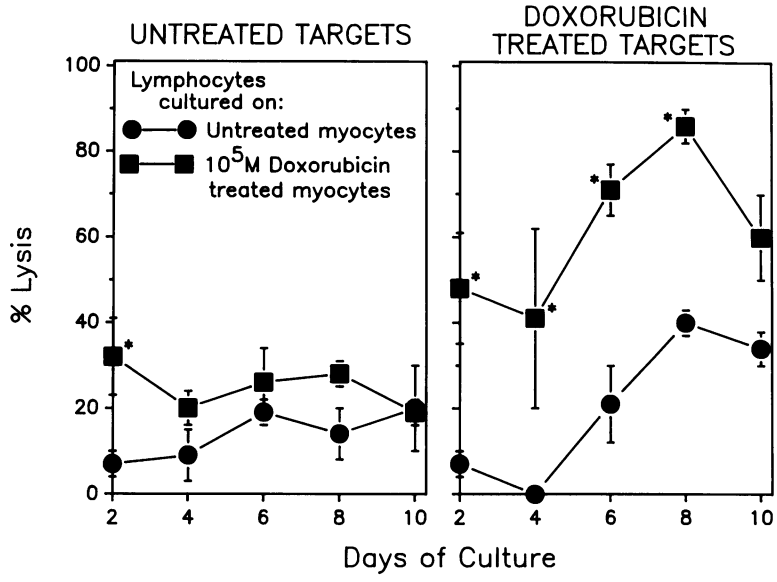

Figure 1. *Kinetics of cytotoxicity generation* in vitro. *Approximately 1 × 10^6 lymphocytes from normal mice were cultured for 2 to 10 days on glutaraldehyde-fixed untreated and doxorubicin-treated syngeneic myocyte monolayers. After culture, surviving lymphocytes were recovered and assayed for cytotoxicity to ^{51}Cr-labeled untreated (left) and doxorubicin-treated (right) myocyte targets. Effector-to-target cell ratio was 100 to 1. Results represent mean percent lysis ± SEM of at least three replicate cultures in one of two experiments. * Cytotoxic activity of lymphocytes cultured on treated myocytes was significantly greater than activity of lymphocytes cultured on untreated myocytes at* P *< 0.05 level.*

1 unless otherwise stated. After incubation the radioactivity in the supernatant and cell pellet were determined using an Intertechnique CG4000 gamma counter (Intertechnique, Lyons, France). Percent ^{51}Cr release was determined using the formula (counts per minute in supernatant)/(counts per minute in supernatant + counts per minute in cells) × 100. Percent lysis was determined by the formula (mean %^{51}Cr release in test group − mean %^{51}Cr release in medium control group)/(maximum %^{51}Cr release by freeze-thaw − mean %^{51}Cr release from medium control) × 100. Percent specific lysis represents the percent lysis by sensitized lymphocytes minus the percent lysis by nonimmune lymphocytes.[10-12] Percent ^{51}Cr release values generally ranged from 33.9% to 56.8% for untreated targets, and from 42.3% to 50.2% for targets treated with 10^{-6} mol/l doxorubicin. Targets treated with 10^{-5} mol/l of the drug showed higher %^{51}Cr release values (60% to 70%), whereas those treated with lower concentrations of the drug more closely approximated release values from untreated targets.

Monoclonal Antibodies

Hybridoma clones GK 1.5 (anti-L3T4), 2.43 (anti-Lyt 2.2), and 30-H12 (anti-Thy 1.2) were obtained from the American Type Culture Collection (ATCC, Bethesda, MD). The cells were initially cultured in DMEM-10% FBS. Subsequently, BALB/c female mice were treated with 0.5 ml pristane intraperitoneally followed 10 days later with 550 R sublethal irradiation and intraperitoneal injection of 10^7 hybridoma cells. Ascites fluid containing the monoclonal antibodies was retrieved 7 to 10 days later and purified by ammonium sulfate precipitation.

Antibody Treatment of Lymphocytes

Approximately 10^7 lymphocytes were incubated with 100 μg monoclonal antibody and 20% rabbit complement at 37°C for 30 minutes. The remaining cells were washed twice with medium, counted by trypan blue exclusion, and adjusted to 10^7 viable cells per milliliter in DMEM-5% FBS.

Statistical Analysis

Differences between groups were statistically evaluated using the Student's *t*-test.

Results

Kinetics of CTL Generation on Untreated and Treated Myocytes

The initial experiments determined whether drug treatment of cultured myocytes produced antigenic alterations capable of stimulating cytolytic cell responses. The myocyte monolayers were treated with 10^{-5} mol/l doxorubicin, or medium alone for 2 hours before glutaraldehyde fixation. Next, nonimmune lymphocytes were cultured on the monolayers for times varying between 2 and 10 days. The lymphocytes were retrieved and assayed for cytotoxicity to myocyte targets (Figure 1). Generally, lymphocytes cultured on untreated stimulating monolayers demonstrated minimal cytotoxicity to untreated targets and only moderate activity to doxorubicin-treated targets. However, cells cultured on doxorubicin-treated myocytes

showed dramatic cytotoxicity to drug-treated targets. Doxorubicin-stimulated lymphocytes were not significantly more cytolytic to untreated targets than lymphocytes cultured on untreated stimulating monolayers. In all cases, cytotoxicity generally peaked between 6 and 8 days of culture.

Next, the stimulating myocytes were treated with varying concentrations of doxorubicin from 10^{-6} to 10^{-8} mol/l for between 0 and 6 hours before glutaraldehyde fixation. Normal lymphocytes were then cultured on the various monolayers for 8 days and surviving cells were assayed for cytotoxicity to drug-treated myocyte targets (Figure 2). This experiment demonstrated that myocytes required 30 minutes' exposure or less to doxorubicin before becoming significantly antigenic to normal syngeneic lymphocytes. Generally, treatment with higher concentrations of doxorubicin appeared to induce greater total antigenicity, as might be expected. Peak stimulation of lymphocytes occurred with myocytes exposed to 10^{-6} mol/l doxorubicin for 4 to 6 hours. Other studies not reported here indicated that stimulation with myocytes exposed to 10^{-5} mol/l doxorubicin generally resembled results obtained with 10^{-6} mol/l concentrations.

Subsequently, the myocytes were treated with varying concentrations of doxorubicin to determine whether this influenced the ability of the cells to act as targets. Effector cells were generated by stimulating normal lymphocytes on glutaraldehyde-fixed myocyte monolayers that had been exposed to 10^{-6} mol/l doxorubicin for 2 hours before fixation. After 8 days, the surviving effectors were retrieved and assayed on myocytes treated with between 10^{-5} and 10^{-8} mol/l of the drug for 2 hours (Figure 3). Controls consisted of targets incubated with normal lymphocytes. Myocytes treated with all concentrations of doxorubicin showed enhanced cytotoxicity with the sensitized cells, although treatment with 10^{-6} mol/l of the drug was optimal. Generally, myocytes were not highly susceptible to lysis with normal lymphocytes, except targets treated with 10^{-5} mol/l doxorubicin. These targets frequently were lysed with both sensitized and unsensitized lymphocytes. However, myocytes treated with these concentrations of doxorubicin also showed elevated spontaneous ^{51}Cr release values, suggesting that this amount of the drug was directly toxic to the targets. Thus, such already damaged targets may be highly susceptible to natural killer cells or macrophage in the lymphocyte population.

The Cytolytic Cell Belongs to the CD8$^+$ T-cell Population

Normal lymphocytes were cultured for 8 days on 10^{-6} mol/l doxorubicin-treated, glutaraldehyde-fixed monolayers as before. The retrieved cells were either untreated, treated with complement alone, or treated with monoclonal antibodies to Thy 1.2 (pan T cell marker), L3T4 (CD4$^+$ cell marker) or Lyt 2.2 (CD8$^+$ cell marker) and complement to deplete selected T cell populations. The lymphocytes were subsequently assayed for cytotoxicity to untreated and doxorubicin-treated myocyte targets (Figure 4). The results clearly indicate that the cytolytic effector cell belongs to the CD8$^+$ T cell population, as depletion of either all T cells or CD8$^+$ cells significantly reduced cytolytic activity, while elimination of CD4$^+$ cells had no effect. Treatment of the lymphocytes with complement alone also slightly reduced cytolytic activity compared with untreated effectors. However, this reduction was not statistically significant.

Tissue Specificity of CTL Lysis

Normal lymphocytes were again stimulated for 8 days on doxorubicin-treated myocyte monolayers. Then sensitized and fresh normal lymphocytes were assayed for cytotoxicity at effector-to-target cell ratios of 25 to 1, 50 to 1, 75 to 1, and 100 to 1 to untreated and doxorubicin-treated cells derived from heart, skin, liver, and kidney (Figure 5). The sensitized lymphocytes showed greatest cytolytic activity to drug-treated kidney and myocyte cultures. Even effector-to-target cell ratios of 25 to 1 could result in cytolysis of 40% or more of the kidney targets in some experiments. Generally, higher effector-to-target cell ratios of 50 to 1 or in the experiment shown here, 75 to 1, were required for significant cytolysis of heart cells. Moderate cytolytic activity also was observed against skin fibroblasts and liver cells but only at the highest effector-to-target cell ratio. The relative sensitivity of the different tissue-derived targets only partially correlated to the concentration of doxorubicin in these cells. The various targets were treated with 10^{-6} mol/l of the drug for 90 minutes, then evaluated by spectrofluorometry for incorporated doxorubicin (Figure 6). Kidney cells that were most sensitive to cytolysis also showed greatest drug accumulation (2.8×10^{-8} mol/l/10^6 cells), whereas skin fibroblasts that were least susceptible to CTL demonstrated significantly ($P < 0.05$) less doxorubicin incorporation (1.0×10^{-8} mol/l/10^6 cells). Interestingly, both heart and liver cells had identical drug concentrations, which were intermediate between those of kidney and fibroblasts.

Discussion

Investigations conducted over the last 2 decades indicate that doxorubicin therapy in experimental animal models also affects the immune system.[9–11,16–19] Usually, these

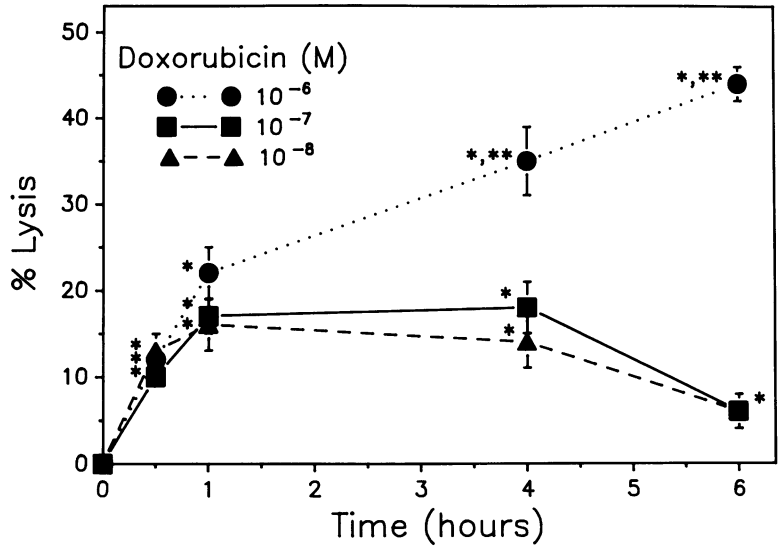

Figure 2. *Kinetics of drug exposure for antigen induction. Myocyte monolayers were exposed to between 10^{-6} to 10^{-8} mol/l (molar) doxorubicin for times ranging from 0 to 6 hours. The monolayers were washed then incubated with 1 × 10^6 lymphocytes for 8 days. The surviving effectors were assayed for cytotoxicity to 10^{-6} md/l doxorubicin-treated myocyte targets at an effector-to-target cell ratio of 100 to 1 and an incubation time of 18 hours. Results represent mean percent lysis ± SEM of triplicate cultures in one of two replicate experiments. * Cytolytic activity with lymphocytes cultured on drug-treated myocytes is significantly greater than activity of lymphocytes cultured on myocytes not exposed to drug at* $P < 0.05$. ** *Cytolytic activity of lymphocytes cultured on myocytes exposed to 10^{-6} mol/l doxorubicin is significantly greater than activity of lymphocytes cultured on myocytes exposed to other drug concentrations at* $P < 0.05$.

studies followed the drug's ability to influence general states of immune competence to a variety of antigenic stimuli, rather than look for more specific responses to drug-induced changes in treated tissues. These more general effects on the immune system may center on macrophages, as doxorubicin therapy both increases phagocytic activity and enriches for immature macrophage in the spleens of treated animals.[16,17] This may simultaneously enhance antigen presentation and macrophage-dependent cytotoxicity. Release of cytokines, including PGE_2 and IL-1, from macrophage in the presence of doxorubicin might additionally immunomodulate antigen-specific responses.[18,19] Other investigators imply that this drug causes the elimination or inhibition of an adherent regulatory cell in the spleen.[20] However, in the present study, we present evidence suggesting that these primarily nonspecific effects on the immune system are not the only immunomodulating characteristics of doxorubicin.

Many drugs can alter cells either directly through intercollation into plasma membranes and interactions with cellular molecules, or indirectly by affecting metabolic processes.[21-23] Because the major purpose of the immune system is to distinguish self from non-self and eliminate the latter, a number of drug-induced alterations might hypothetically initiate immunologic reactions. Clearly, penicillin and chemicals such as tri- or dinitrophenol act as potent haptens.[22,23] When bound with carrier proteins, they stimulate strong and, in the case of penicillin, potentially dangerous immune reactions.[24] Doxorubicin also intercollates into the cell membranes and alters cellular metabolism.[25-27] As shown in the present communication, one or both of these interactions must induce antigenic

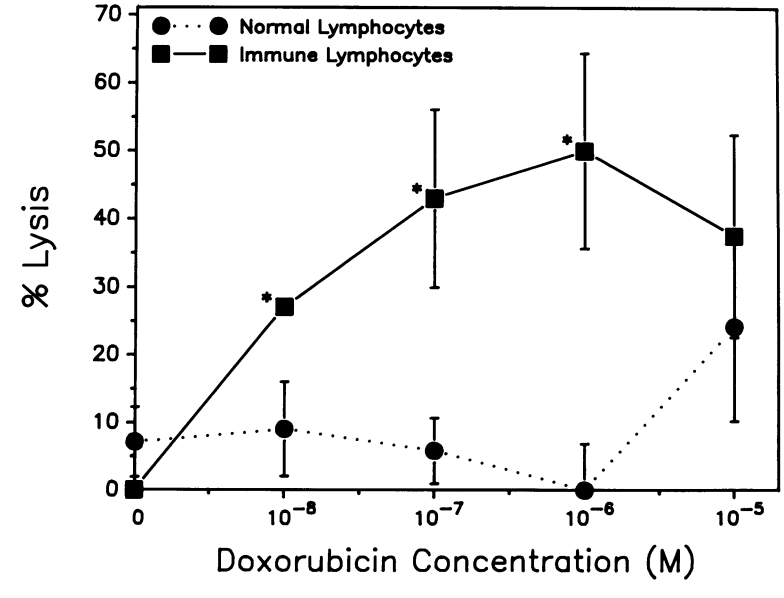

Figure 3. *Effect of doxorubicin concentration on target cell antigenicity. Lymphocytes from normal mice were stimulated on 10^{-6} mol/l doxorubicin-treated myocyte monolayers for 8 days. Surviving immune lymhocytes were recovered and assayed for cytotoxicity at a 100 to 1 effector to target cell ratio on myocyte targets treated with different concentrations of doxorubicin. Normal (nonsensitized) lymphocytes were also assayed at 100 to 1 effector-to-target cell ratio on the myocyte targets. Results represent mean percent lysis of at least triplicate cultures for each point ± SEM in one of three replicate experiments. * Cytotoxicity is significantly greater than with normal lymphocytes at* $P < 0.05$. *No significant differences were observed in cytolytic activity of immune lymphocytes assayed on targets exposed to 10^{-8}, 10^{-7}, or 10^{-6} mol/l doxorubicin.*

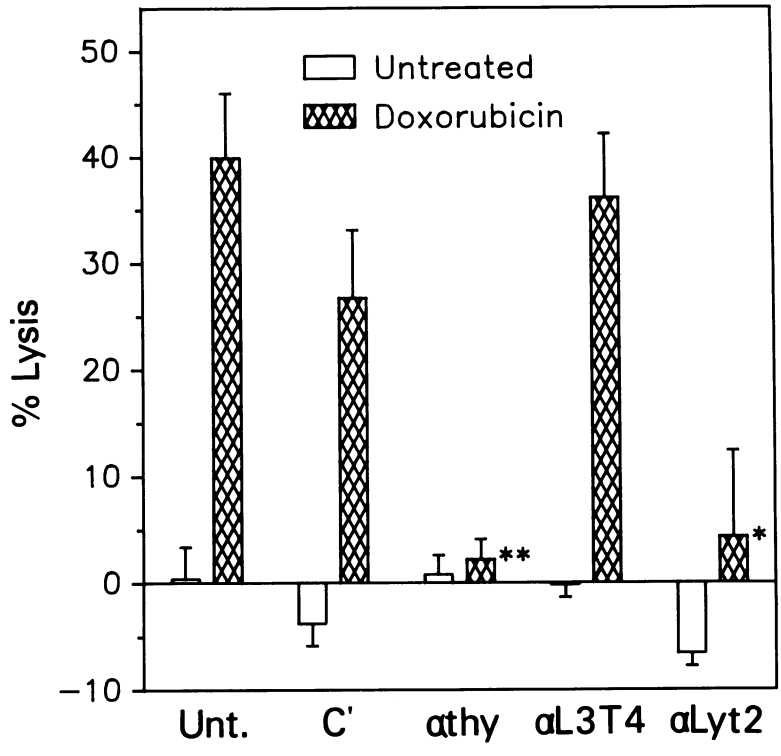

Figure 4. *Cytotoxicity is mediated by CD8⁺ T cells. Sensitized lymphocytes were produced by culturing lymphocytes from normal mice for 8 days on myocyte monolayers treated with 10^{-5} mol/l doxorubicin for 2 hours before glutaraldehyde fixation. The surviving lymphocytes were untreated, treated with 20% rabbit complement alone, or treated with rabbit complement and 100 μg/ml monoclonal antibody to either all T cells (anti-Thy 1.2), CD4⁺ (anti-L3T4) or CD8⁺ (anti-Lyt 2) T cells. The lymphocytes were subsequently assayed for cytotoxicity to either untreated or 10^{-6} mol/l doxorubicin-treated myocyte targets at a 100 to 1 effector-to-target cell ratio and an incubation period of 18 hours. Results represent mean percent lysis of six replicate cultures in one of two experiments. * Cytotoxicity of antibody and/or complement-treated lymphocytes is significantly less than untreated lymphocytes at* $P < 0.05$.

alterations capable of stimulating CD8⁺ CTL responses to drug-treated cells. How doxorubicin causes alterations recognizable to the immune system remains unclear. The antigenic change might result from membrane effects of the drug. This conclusion depends primarily on the demonstration that exposure of myocytes to doxorubicin for as little as 30 minutes can produce alterations triggering immune responses. A second possibility, however, is that the drug-related antigen results from metabolic alteration of the target cells. We have shown that viruses that inhibit cellular metabolism in myocytes produce acceptable targets for CTL.[28] In the present study, sensitivity of cells to CTL-mediated lysis does not completely correlate to the concentration of drug incorporated. True, kidney-derived cells are most susceptible to cytolysis and contain the most doxorubicin, while skin fibroblasts are least sensitive and contain the least drug. However, myocytes are nearly as susceptible to lysis as kidney cells, yet contain concentrations of doxorubicin identical to liver-derived cells, which are relatively resistant to lysis. Thus, other factors besides total concentration of the drug in the target cells must influence either antigenicity or lysogenicity. The concentrations of doxorubicin found in each of these tissue cell types was somewhat surprising. Liver, kidney, and heart are among the organs/tissues of greatest concentration of the drug *in vivo*.[3] It is not surprising that these

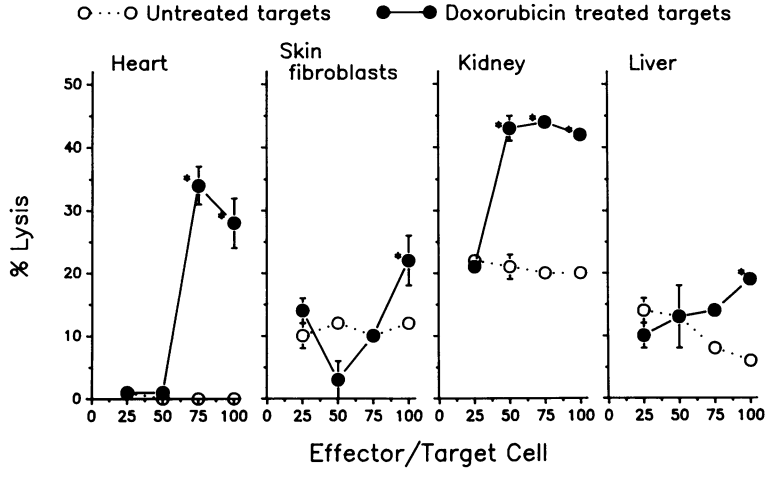

Figure 5. *Cytotoxicity to target cells of different tissue origin. Immune lymphocytes were obtained by culturing normal lymphocytes for 8 days on myocyte monolayers treated with 10^{-6} mol/l doxorubicin. Immune lymphocytes were assayed for cytotoxicity at effector-to-target cell ratios ranging from 25 to 1 to 100 to 1 on untreated and 10^{-6} mol/l doxorubicin-treated target cells derived from neonatal murine heart, skin, liver, and kidney. Results represent mean percentage specific lysis of triplicate cultures ± SEM in one of three experiments. * Cytotoxicity on doxorubicin-treated targets significantly exceeded cytotoxicity to untreated targets at* $P < 0.05$.

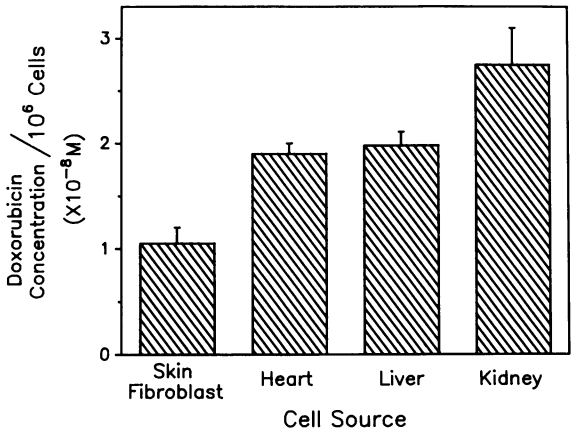

Figure 6. *Doxorubicin concentrations in target cells derived from different tissues. Cells were derived from heart, liver, skin, and kidney as target cells for the previous figure. Identical cell monolayers were treated with 10^{-6} mol/l doxorubicin, washed, and evaluated for incorporated drug by fluorescence at 470 nm. Results represent mean ± SEM of two replicate cultures for each cell types. Statistical differences were found in drug concentrations/10^6 cells as follows: kidney > heart, liver > skin at* $P < 0.05$.

cells might contain more of the drug *in vitro* as well, but one might have expected that the myocytes, with their high levels of cardiolipin, should have contained the most drug. However, cardiolipin concentrations in neonatally derived cells may not be equivalent to that in fully differentiated adult cells.

Drug treatment might also make the myocytes more susceptible to lysis by nonspecific cytolytic effectors. Considering the usual toxicity of doxorubicin, it is reasonable or even probable that this agent makes targets that would normally be resistant to macrophage and natural killer cell-type killing significantly more susceptible to both mediators. While this possibility cannot be excluded presently, it seems unlikely. The drug treatment protocol exposes target cells to doxorubicin for limited times, after which the unincorporated drug is removed. Although much of the drug undoubtedly remains in the cells, ^{51}Cr release from the treated targets is little different from untreated targets at any but the highest (10^{-5} mol/l) doxorubicin concentrations used. This result probably indicates that the toxicity and antigenic effects of the drug are different. Furthermore, $CD8^+$ CTL most often represent antigen-specific effectors rather than nonantigen-specific cytolytic cells.

One question might be whether these *in vitro* observations and the CTL activity generated to doxorubicin-treated cells in any way reflects pathogenic mechanisms of tissue toxicity *in vivo*. Certainly, most investigators would undoubtedly credit the known cytolytic capacity and metabolic effects of the drug for much of the toxicity observed. This report does not dispute that the drug itself is a potent agent capable of much cellular damage. However, the ability of doxorubicin-treated cells to trigger immunologic responses could contribute, at least minimally, to the toxicity associated with therapy. It might additionally have a positive effect in controlling tumors, especially those that are normally weakly immunogenic. Exposure to doxorubicin could result in a two-pronged attack. First, the natural toxicity of the drug will eliminate many tumor cells directly. Secondly, by increasing the antigenicity of the remaining cells not receiving a lethal dose of the drug, antitumor immunity may become more effective.

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