ENERGY METABOLISM AND T-CELL-MEDIATED CYTOLYSIS

I. Synergism between Inhibitors of Respiration and Glycolysis*

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Although it has been recognized for some time that cytolytic thymus-derived lymphocytes (CTL)[1] are generated in the course of cell-mediated immune responses to allografts and many virus-induced tumors (1, 2), the mechanism of T-cell-mediated cytolysis at the molecular level remains unclear. It is generally accepted, however, that cytolysis is temperature and energy dependent, involves direct contact between metabolically active CTL and target cells, requires divalent cations, and is probably independent of *de novo* synthesis of macromolecules (for review see references 3–5). A number of other inhibitors of cytolysis have been described (e.g., cytochalasin B and dimethyl sulfoxide) but the molecular basis of their effect on CTL is unknown.

Recent studies in several laboratories have examined the energy dependence of cytolysis in some detail (6–8). These reports have confirmed that antagonists of energy metabolism inhibit cytolysis, and further suggested that some degree of energy is required both for the binding of CTL to target cells and for the cytolytic event itself. Despite these results, however, the relative roles of respiration and glycolysis in T-cell-mediated cytolysis have not been clearly defined, particularly since the inhibitors which have been commonly used may lead to secondary effects on CTL energy metabolism. We have therefore reexamined the energy requirements for cytolysis with particular emphasis on oxygen and glucose, the primary metabolites of cellular energy production. The results of these studies suggest that cytolysis can proceed in the absence of oxygen (<0.2 μM) or in very low concentrations of exogenous glucose (<5 μM). Inhibition of glycolysis in the absence of oxygen or inhibition of respiration in the absence of glucose, however, resulted in a dramatic and reversible inhibition of cytolytic function.

Materials and Methods

Mice. Adult mice of the inbred strains C57BL/6 and DBA/2 were obtained from The Jackson Laboratory, Bar Harbor, Maine.

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[1] *Abbreviations used in this paper:* ADCC, antibody-dependent cell-mediated cytotoxicity; CTL, cytolytic thymus-derived lymphocytes; 2-DG, 2-deoxy-D-glucose; FCS, fetal calf serum; MLC, mixed leukocyte culture; SA, sodium azide; 5-SH-G, 5-thio-D-glucose.

Reagents. Sodium azide (SA), 2,4-dinitrophenol (DNP), D-glucose, 2-deoxy-D-glucose (2-DG), and 3-*O*-methyl-α-D-glucopyranoside (3-*O*-methylglucose) were obtained from the Sigma Chemical Co., St. Louis, Mo. 5-thio-D-glucose (5-SH-G) was obtained from Pfanstiehl Labs., Inc., Waukegan, Ill. DNP was dissolved in 0.1 N sodium hydroxide and diluted with phosphate-buffered saline to six times the desired final concentration. All other reagents were dissolved in phosphate-buffered saline and likewise diluted.

Generation of CTL. CTL were generated in mixed leukocyte cultures (MLC) by a slight modification of a technique described in detail elsewhere (9). Briefly, 25×10^6 C57BL/6 spleen cells were mixed with an equal number of irradiated (1,000 rads) DBA/2 spleen cells in 25 cm^2 tissue culture flasks in 20 ml of Eagle's minimum essential medium (MEM) supplemented with nonessential amino acids, 2% (vol/vol) fetal calf serum (FCS), and 5×10^{-5} M 2-mercaptoethanol. Flasks were incubated upright for 4–6 days in a humidified atmosphere of 5% CO_2 in air. At the end of the incubation period, cells recovered from the flasks were washed and resuspended in appropriate medium for the particular assay procedure (see below). The number of viable (i.e., trypan blue excluding) cells was then adjusted as required for the cytolytic assay.

Target Cells. P-815 mastocytoma cells were maintained in vitro and labeled with $Na_2{}^{51}CrO_4$ as described in detail elsewhere (9). Labeled cells were washed three times in appropriate medium and resuspended to a concentration of 5×10^4 cells/ml.

Cytolytic Assay. The cytolytic assay was as described previously (9) with the following modifications.

For anaerobic studies, both MLC cells and labeled P-815 mastocytoma cells were resuspended at 0°C in MEM supplemented with 5% (vol/vol) FCS and 10 mM HEPES buffer. Various numbers of MLC cells in 0.2 ml were then mixed on ice with 0.2 ml (1×10^4) labeled P-815 target cells in 12 × 75-mm glass tubes (Fisher Scientific Co. Ltd., Toronto, Canada). Spontaneous release controls contained labeled target cells but no lymphocytes. The tubes were then placed in airtight aluminum chambers which were sequentially evacuated and refilled with an atmosphere of 95% nitrogen and 5% CO_2 over a period of 4.5 h. The chambers were maintained at 0°C throughout this procedure to prevent interactions between CTL and target cells (3–5). Petri dishes containing 0.1 M sodium dithionite dissolved in 0.5 N sodium hydroxide and 0.5 N sodium bicarbonate were added to chambers which were to remain hypoxic. This solution removes the last traces of oxygen from the atmosphere. A detailed description of the chambers and of the evacuation technique has been published elsewhere (10, 11). During the final 30 min of the degassing procedure, an atmosphere of 95% air and 5% CO_2 was admitted into those chambers to be used as aerobic controls. Then both the aerobic and anaerobic chambers were placed in a water bath at 37°C for 2 h to allow cytolysis to occur. At the end of this incubation period, the chambers were again cooled to 4°C and opened. The tubes were centrifuged at 500 *g* for 5 min, and half the supernate was decanted and assessed for radioactivity in a well-type scintillation counter. Cytolysis was calculated as described below.

For assays involving low concentrations of exogenous glucose, both MLC cells and labeled P-815 mastocytoma cells were washed three times in glucose-free Eagle's basal medium containing 2% (vol/vol) dialyzed FCS and 10 mM HEPES buffer. The glucose content of this medium [measured by the glucose oxidase technique (12)] was found to be <5 μM. MLC cells (1×10^5 in 0.2 ml) were then mixed with ^{51}Cr-labeled mastocytoma cells (1×10^4 in 0.2 ml) in round-bottomed 10 × 65 mm plastic tubes (Luckham Ltd., Surrey, U.K.) in the presence or absence of various concentrations of glucose and/or other anti-metabolites as described in the text. All mixtures were brought up to a final vol of 0.6 ml with glucose-free medium. Tubes were centrifuged to promote cell contact (70 *g* for 2 min) and incubated at 37°C for 1.5 h in a humidified atmosphere at 5% CO_2 in air. At the end of the incubation period, tubes were centrifuged and assayed for ^{51}Cr as above.

Calculation of Cytolysis. Specific lysis in all cases was calculated according to the formula:

$$\text{Percent specific } {}^{51}\text{Cr release} = [(\text{experimental cpm} - \text{spontaneous cpm})/(\text{maximal cpm} - \text{spontaneous cpm})] \times 100.$$

Spontaneous release was always determined in the presence of the appropriate dilution of inhibitor. Unless otherwise stated, spontaneous release in the presence of inhibitors did not exceed control spontaneous release by more than 5%. Maximal release was determined by incubating target cells for the duration of the assay in 0.5 N hydrochloric acid; values so obtained were generally 85–90% of the total counts per minute incorporated.

Results

Dependence of Cytolysis upon Exogenous Glucose. The question of whether exogenous glucose is absolutely required for T-cell-mediated cytolysis has been investigated by several workers (13–15). A major problem in any experimental approach to this question is the dependence of cytolysis upon serum, which normally contains glucose at concentrations of the order of 1 mg/ml (5.5×10^{-3} M). To partially circumvent this problem, we have used extensively dialyzed FCS so that the final concentration of glucose in the assay medium was $<5 \times 10^{-6}$ M. The results of 20 consecutive experiments comparing cytolysis in this low glucose medium with normal glucose-supplemented medium are summarized in Table I. It can be seen that significant cytolysis was observed in all cases in low glucose medium, and the average reduction in cytolysis in the absence of glucose was only 10%. These studies indicated that very low concentrations of exogenous glucose were sufficient to support cytolysis and provided no evidence for its obligatory role.

Dependence of Cytolysis upon Oxygen. Although metabolic inhibitors of cellular respiration have been found to inhibit T-cell-mediated cytolysis in several instances (6–8), few, if any, studies have dealt with the dependence of cytolysis upon oxygen. We have approached this question directly by assaying cytolysis in specially designed chambers in which the oxygen concentration can be maintained below 10^{-8} M (10, 11). The results of seven consecutive experiments comparing cytolysis under aerobic and anaerobic conditions are summarized in Table II. Although specific ^{51}Cr release was slightly reduced in the absence of oxygen in five of seven experiments, it is clear that significant cytolysis occurred in the absence of cellular respiration in every case. In other experiments, incubation of MLC cells in the absence of target cells for up to 3 h at 37°C under anaerobic conditions was found to have no effect on their ability to damage target cells in a subsequent assay performed under aerobic conditions (data not shown), indicating that any effects of extreme hypoxia on CTL function were completely reversible. These experiments indicated that aerobic energy metabolism was not obligatory for T-cell-mediated cytolysis; however, optimal cytolysis was dependent upon the presence of oxygen in most experiments.

Effect of Glucose Analogues on Cytolysis in the Absence of Oxygen. Since cytolysis was not dramatically inhibited in the absence of glucose or oxygen individually, it was of interest to investigate the combined effect of inhibition of respiration and glycolysis on CTL. Since spontaneous release values were significantly elevated under conditions where both glucose and oxygen were absent (data not shown), we approached this question by utilizing metabolic inhibitors of glycolysis and respiration in the presence or absence of oxygen and glucose, respectively. The effect of the glucose analogues 2-DG, 5-SH-G, and 3-*O*-methylglucose on cytolysis under anaerobic conditions was initially investigated. The experimental design for these studies was the same as for the other anaerobic experiments except that the analogues were added to mixtures of CTL and target cells at the same molar concentration as glucose. The results of two experiments of this type (Table III) indicated that the addition of 5 mM 2-DG or 5-SH-G completely inhibited cytolysis in the absence of oxygen, whereas they

TABLE I
*T-Cell-Mediated Cytolysis in Low Concentrations of Exogenous Glucose**

Exp.	Percent specific ^{51}Cr release		
	$<5 \times 10^{-6}$ M glucose (A)	5×10^{-3} M glucose (B)	Ratio (A/B)
1	61	61	1.00
2	21	41	0.51
3	23	29	0.79
4	50	44	1.14
5	57	56	1.02
6	35	53	0.66
7	48	50	0.96
8	69	65	1.06
9	73	74	0.99
10	45	49	0.92
11	73	60	1.22
12	48	53	0.91
13	40	61	0.66
14	23	40	0.58
15	16	17	0.94
16	29	32	0.91
17	81	83	0.98
18	67	76	0.88
19	35	37	0.95
20	45	56	0.80
		Mean ± SD	0.90 ± 0.18

* MLC cells ($0.5–2 \times 10^5$) in low glucose medium were assayed for cytotoxicity on 10^4 ^{51}Cr-labeled mastocytoma cells in the presence or absence of 5×10^{-3} M glucose.

TABLE II
*Effect of Extreme Hypoxia on T-Cell-Mediated Cytolysis**

Exp.	Percent specific ^{51}Cr release					
	Aerobic conditions			Hypoxic conditions		
	30:1‡	10:1	3:1	30:1	10:1	3:1
1	38	20	7	32	9	0
2	63	30	24	62	38	12
3	46	30	8	24	15	4
4	49	26	10	51	32	10
5	39	ND§	ND	17	ND	ND
6	67	41	18	50	21	5
7	65	ND	ND	44	ND	ND

* Various numbers of MLC cells were mixed at 4°C with 10^4 ^{51}Cr-labeled mastocytoma cells and rendered hypoxic in special chambers as described in detail in the text. Control chambers were then reoxygenated (aerobic conditions), and cytotoxicity was assessed in a 2–3 h assay at 37°C. The assay medium contained 5 mM glucose.

‡ Lymphocyte:target cell ratio.

§ ND, not done.

TABLE III

*Effect of Glucose Analogues on Cytolysis in the Presence or Absence of Oxygen**

Inhibitor added	Percent specific ^{51}Cr release	
	Aerobic conditions	Hypoxic conditions
None	41;65	21;44
2-DG (5×10^{-3} M)	56;48	1;4
5-SH-G (5×10^{-3} M)	49;59	0;0
3-*O*-methylglucose (5×10^{-3} M)	ND‡;48	ND;41

* Mixtures of MLC cells (2×10^5) and ^{51}Cr-labeled mastocytoma cells (1×10^4) were rendered hypoxic and assayed for cytolysis as described for Table II. The assay medium contained 5×10^{-3} M glucose in addition to the indicated glucose analogues. Data are from two separate experiments.

‡ ND, not done.

had no effect under aerobic conditions. In contrast, 3-*O*-methylglucose failed to inhibit cytolysis under either anaerobic or aerobic conditions.

Effect of Respiratory Antagonists on Cytolysis in the Absence of Glucose. In parallel studies, the effect of respiratory antagonists on cytolysis in the absence of glucose was investigated. In particular, various concentrations of SA and DNP were added to mixtures of CTL and target cells in low glucose medium or in the same medium supplemented with 5 mM glucose. The results of a typical experiment (Fig. 1) indicated that inhibition of cytolysis by these agents was highly dependent upon glucose concentration. 25-fold higher concentrations of DNP were required to inhibit cytolysis when glucose was present, and SA only inhibited cytolysis in the low glucose medium.

Synergism between Inhibitors of Respiration and Glycolysis. The combined effect of inhibitors of respiration and glycolysis was also studied in several experiments. In agreement with the results presented above, neither 10 mM SA nor 5 mM 2-DG alone inhibited cytolysis to an appreciable extent in the presence of 5 mM glucose (Table IV). However, the simultaneous presence of both inhibitors resulted in a complete inhibition of cytolysis. More detailed experiments using the other glucose analogues under the same assay conditions (Fig. 2) established that 5-SH-G was likewise effective in inhibiting cytolysis in the presence of 10 mM SA, whereas 3-*O*-methylglucose was ineffective. On a molar basis, 5-SH-G was slightly more inhibitory than 2-DG.

Reversibility of Inhibition of Cytolysis. The experiments described above indicated that T-cell-mediated cytolysis was strongly inhibited when antagonists of cellular respiration and glycolysis were simultaneously present. In order to determine whether this inhibition could be specifically attributed to either the CTL or the target cells, MLC cells or labeled mastocytoma cells were incubated separately with SA in combination with the glucose analogues 2-DG and 5-SH-G as described in Table IV and Fig. 2. Treated cells were then washed and assayed for cytotoxicity using appropriate numbers of untreated MLC cells or target cells. The results of such an analysis (Table V) indicated that the effect of the inhibitors was completely reversible after washing of either the MLC cells or the target cells. In similar experiments, the reversibility of the inhibitory

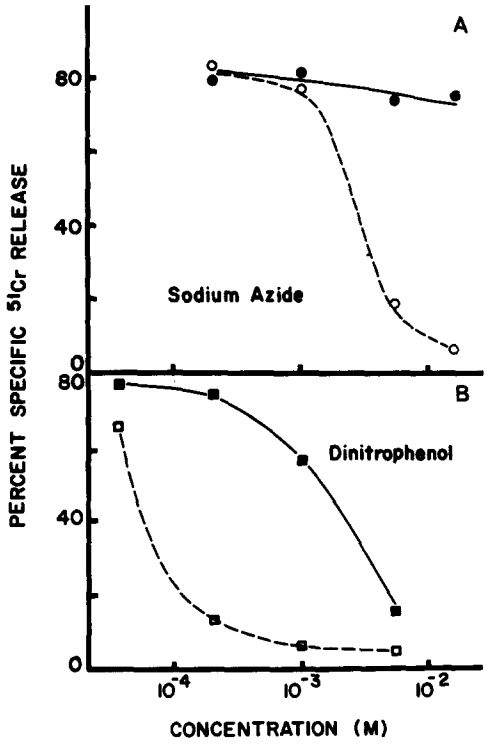

FIG. 1. Effect of SA and DNP on cytolysis in the presence or absence of glucose. Various concentrations of SA (Fig. 1 A) were added to mixtures of MLC cells (1×10^5) and ^{51}Cr-labeled mastocytoma cells (1×10^4) in low glucose medium and cytolysis was assessed with (●—●) or without (○---○) the addition of 5×10^{-3} M glucose. Similarly, the effect of DNP (Fig. 1 B) was assessed in the presence (■—■) or absence (□---□) of 5×10^{-3} M glucose. Cytolysis in the absence of inhibitor was 81% in low glucose medium and 83% in glucose-supplemented medium.

TABLE IV
*Synergistic Effect of SA and 2-DG on Cytolysis**

Inhibitor added	Percent specific ^{51}Cr release	Percent inhibition
None	65	–
SA (10^{-2} M)	55	15
2-DG (5×10^{-3} M)	63	3
SA + 2-DG	1.6	98

* MLC cells (10^5) were assayed for cytotoxicity on ^{51}Cr-labeled mastocytoma cells (10^4) in normal (glucose-containing) assay medium in the presence of the indicated concentration of inhibitor(s).

procedures described in Table III and Fig. 1 was also confirmed (data not shown). Hence it is not possible on the basis of these data to directly establish whether the energy requirement for cytolysis pertains to CTL or target cells (or both).

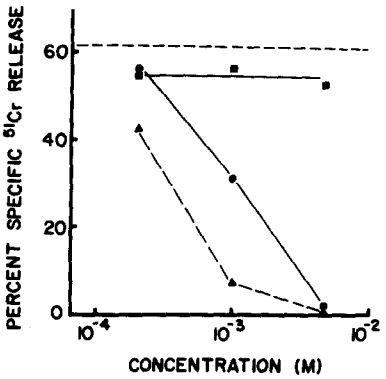

FIG. 2. Effect of glucose analogues on cytolysis in the presence of SA. MLC cells (10^5) in glucose-containing medium were assayed for cytotoxicity on ^{51}Cr-labeled mastocytoma cells (10^4) in the presence of 10^{-2} M SA alone (---) or supplemented with various concentrations of 2-DG (●——●), 5-SH-G (▲---▲), or 3-*O*-methylglucose (■—■). Cytolysis in the absence of inhibitor(s) was 81% and was not affected by the glucose analogues in the absence of SA.

TABLE V

*Reversibility of Combined Effect of Glucose Analogues and SA on CTL and Target Cells**

Pretreatment procedure		Percent specific ^{51}Cr release			
Cells	Inhibitor(s)	30:1‡	10:1	3:1	1:1
Exp. 1					
MLC	None	88	52	21	7
MLC	SA(10 mM)	87	52	19	8
MLC	SA(10 mM) + 2-DG(5 mM)	89	53	20	9
MLC	SA(10 mM) + 5-SH-G(5 mM)	88	50	16	6
Exp. 2					
Mastocytoma	None	66	36	14	5
Mastocytoma	SA(10 mM)	67	36	15	5
Mastocytoma	SA(10 mM) + 2-DG(5 mM)	67	32	12	3
Mastocytoma	SA(10 mM) + 5-SH-G(5 mM)	69	38	16	6

* MLC cells (10^6/ml) (exp. 1) or ^{51}Cr-labeled mastocytoma cells (2×10^5/ml) (exp. 2) were incubated with inhibitor(s) in normal (glucose-containing) assay medium for 90 min at 37°C. Treated cells were then washed and assayed for cytotoxicity using appropriate numbers of untreated target cells or MLC cells, respectively.

‡ Lymphocyte:target cell ratio.

Discussion

The results presented in this communication provide strong evidence that the energy requirements for T-cell-mediated cytolysis can be supplied by either oxidative or glycolytic energy pathways. In particular, cytolysis was only slightly reduced in the absence of exogenous glucose or oxygen, and inhibitors of oxygen and glucose utilization were ineffective (or effective only at high concentration) when used separately to block cytolysis. On the other hand, the simultaneous inhibition of both respiration and glycolysis resulted in a dramatic inhibition of cytolysis under all conditions tested, confirming the over-all energy dependence of the phenomenon (1, 3–8).

Direct evidence that cytolysis is not absolutely dependent on cellular respiration was provided by experiments in which the cytolytic assay was performed under conditions of extreme hypoxia (Table II). These experiments were carried out in specially designed chambers in which the concentration of oxygen can be maintained at very low levels (approximately 10 ppm or 10^{-8} M) both in the atmosphere and, under appropriate conditions, in the medium itself (10, 11, 16). For the particular geometry used in this study, the level of oxygen present in the assay medium was estimated to be $<2 \times 10^{-7}$ M on the basis of measurements of the oxygen dependence of the radiation response of tissue culture cells irradiated under identical experimental conditions (C. J. Koch, unpublished data). Such studies make it clear that aerobic energy metabolism is not obligatory for the expression of cell-mediated cytolysis.

The results obtained with the respiratory antagonists SA and DNP confirm previous reports documenting the reversible inhibitory effect of these agents on cytolysis (6, 7). However, the present study further demonstrates that inhibition of cytolysis by SA and DNP is strongly dependent upon the amount of exogenous glucose present (Fig. 1). In particular, the inhibitory effect of SA on cytolysis in low glucose medium was reversed by the addition of 5 mM glucose, and the concentration of DNP required to inhibit cytolysis increased 25-fold when 5 mM glucose was added to the assay medium. With regard to the latter effect, it has been shown elsewhere (17) that DNP uncouples oxidative phosphorylation in lymphocytes at the same concentration (2×10^{-4} M) as was required to inhibit cytolysis in the absence of glucose in these studies. Therefore it seems likely that the inhibitory effect of high concentrations of DNP on cytolysis in the presence of glucose may be related to secondary effects on CTL energy metabolism. If this is so, the results obtained with SA and DNP confirm the anaerobic studies, indicating that oxidative energy production is not required for T-cell-mediated cytolysis.

In agreement with other recent reports (14, 15), the present experiments likewise do not support any obligatory role for glycolysis in T-cell-mediated cytolysis. Consistent with this view was the failure to observe significantly reduced cytolysis in medium containing very low concentrations (<5 μM) of exogenous glucose (Table I), and the inability of the glycolytic inhibitors 2-DG and 5-SH-G to inhibit cytolysis in glucose-containing medium unless cellular respiration was simultaneously inhibited (Table III, Fig. 2). Although the assay medium used in these studies was sufficiently depleted of glucose to preclude significant consumption by lymphocytes (see references 17–19), it should be noted that rigorous depletion of glucose was not achieved. Moreover, the possibility that glycolysis could proceed for the limited duration of the assay via the breakdown of intracellular glycogen reserves or by amino acid catabolism cannot be excluded by the present data, although inhibition of these processes by the glucose analogues 2-DG and 5-SH-G might be expected (*vide infra*).

Although cytolysis was apparently not absolutely dependent on either respiration or glycolysis in these studies, combined inhibition of the two energy pathways resulted in a dramatic inhibition of CTL function in all cases. This synergistic inhibition was demonstrated by using respiratory inhibitors (SA and DNP) in the absence of glucose (Fig. 1) and by using glycolytic inhibitors (2-DG

and 5-SH-G) in the absence of oxygen (Table III). Combinations of the two classes of inhibitors were also very effective (Table IV, Fig. 2). Because CTL represent a subpopulation of unknown frequency in the relatively heterogeneous MLC population (1), no direct attempt was made to correlate inhibition of cytolysis with reductions in the concentration of various energy intermediates (such as ATP) in these studies. However, in view of the reversibility of the combinations of inhibitors tested, it seems clear that cytolysis requires more energy than is necessary for the survival of CTL.

An interesting feature of the combined inhibition studies was the ability of 2-DG and 5-SH-G, but not 3-*O*-methylglucose, to inhibit cytolysis under conditions where respiration was strongly inhibited (Table III, Fig. 2). In this context, it is known that 2-DG and 5-SH-G (and their phosphorylated derivatives) strongly inhibit glycolysis in mammalian cells via competitive inhibition of key enzymes such as hexokinase, phosphoglucoisomerase, and phosphoglucomutase (20–22). In fact, complete inhibition of glycolysis (as defined by lactate production) has been observed in the presence of 2-DG in isolated guinea pig lymph node cells (23) and in human lymphocytes stimulated by phytohemagglutinin (24). 3-*O*-methylglucose, on the other hand, is a competitive inhibitor of glucose transport which is not phosphorylated or further metabolized in mammalian cells (25, 26). In view of these findings, the failure of 3-*O*-methylglucose to inhibit cytolysis in the absence of respiration may indicate that effective inhibition of glycolytic energy production in CTL is dependent upon interference with the phosphorylation and/or further metabolism of glucose. Such a finding might be expected if glucose were being synthesized endogenously by CTL under these conditions. In any case, the relationship between glucose metabolism and CTL function will be explored in greater detail in the accompanying communication (27).

Although the foregoing discussion has concentrated on the possible consequences of inhibition of energy metabolism in CTL, it should be noted that metabolic effects on the susceptibility of the target cells cannot be formally excluded. In particular, the complete reversibility of the inhibition after pretreatment and washing of CTL or target cells (Table V) is consistent with an inhibitory mechanism which is directed at either CTL or target cells (or both). Unfortunately, the absolute dependence of cytolysis upon cell contact (1, 3–5) precludes a more direct experimental approach to this question.

In addition to considerations of cellular energy metabolism, the demonstration that CTL can lyse target cells at extremely low oxygen concentrations is of interest insofar as the putative role of such cells in tumor immunity is concerned. In this regard, it is known that solid tumors contain radiobiologically hypoxic regions in which the level of oxygen, while not known with certainty, has been estimated at 1,000 ppm (28, 29). Hence the demonstration that T-cell-mediated cytolysis can occur (at least in a short-term assay) at oxygen concentrations which are lower than those likely to be encountered in the tumor microenvironment in vivo does not preclude a possible role for these cells in tumor immunity. Studies are currently in progress to assess the ability of CTL to survive for extended periods of time under conditions of moderate and extreme hypoxia.

Finally, in a recent study, Trinchieri and de Marchi have also investigated

the relative contributions of oxidative and glycolytic energy metabolism in an antibody-dependent cell-mediated cytotoxicity (ADCC) system using human peripheral blood leukocytes as a source of effector cells (30). Their findings using combinations of energy inhibitors suggested that ADCC was likewise not dependent on a specific energy pathway, but rather depended on the maintenance of certain minimal levels of total energy production. Although ADCC is operationally distinct from the system described here, the observed similarities in energy dependence extend the mechanistic analogies already noted between the two model systems (8, 31, 32).

Summary

The energy requirements for T-cell-mediated cytolysis have been investigated. Cytolytic thymus-derived lymphocytes (CTL) were generated in vitro in mixed leukocyte cultures and assayed for cytotoxicity on ^{51}Cr-labeled mastocytoma target cells. Cytolysis was only slightly reduced in the absence of exogenous glucose (<5 μM) or under conditions of extreme hypoxia (<0.2 μM oxygen). Furthermore, neither the glucose analogues 2-deoxy-D-glucose and 5-thio-D-glucose nor the respiratory antagonists sodium azide and 2,4-dinitrophenol were very effective inhibitors of cytolysis when used individually. However, these glucose analogues were highly effective in inhibiting cytolysis in the absence of oxygen, and the respiratory antagonists inhibited cytolysis to a much greater extent in the absence of glucose. In addition, synergistic effects were observed when the glycolytic and respiratory inhibitors were combined. Taken together, these results indicate that T-cell-mediated cytolysis is an energy-dependent process which can be supported by either oxidative or glycolytic energy pathways.

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