Lymphocyte Activation and Phospholipid Pathways

31P MAGNETIC RESONANCE STUDIES*

(Received for publication, July 16, 1990)

Ofer Kaplan‡ and Jack S. Cohen§

From the Medicine Branch, Division of Cancer Treatment, National Cancer Institute, Bethesda, Maryland 20892

31P NMR spectra of perfused lymphocytes, embedded in alginate capsules and activated by interleukin-2, were remarkably different from those of control lymphocytes. The main differences were the appearance and gradual increase in phosphodiester signals, glycerophosphocholine and glycerophosphoethanolamine. These metabolic changes also occurred following perfusion with phorbol ester and after incubation with phytohemagglutinin (PHA) and were not dependent on a special growth medium. Nifedipine, a calcium channel blocking drug, inhibited the effects of phytohemagglutinin, but not of interleukin-2. There were no NMR spectral differences between peripheral lymphocytes, stimulated for 3 weeks, and tumor-infiltrating lymphocytes. Thus, sustained accelerated turnover of phosphatidylcholine and phosphatidylethanolamine is an inherent feature of the activation process. 31P NMR spectra of lymphocytes are characterized by a low signal of phosphocholine. Perfusion studies with high concentrations of choline and the use of dapsone, an inhibitor of cytidylyltransferase, indicated that choline kinase plays a key role in regulating phosphatidylcholine synthesis in human lymphocytes.

While early events of cellular activation have been thoroughly investigated and are well documented (see reviews 1, 2), subsequent metabolic processes are still ill defined. Late biochemical changes in lymphocyte activation may be of clinical relevance in adoptive immunotherapy, utilizing expanded cultures of tumor-infiltrating lymphocytes (TIL) grown for prolonged periods in the presence of interleukin-2 (IL-2) (3, 4). Lymphocyte activation is initiated by enhanced breakdown of phosphatidylinositol into inositol triphosphate and diacylglycerol (5, 6). These second messengers subsequently induce elevation of cytosolic calcium and activation of protein kinase C, respectively. Activated protein kinase C enhances phosphorylation of various cellular proteins and transcription of several genes, including the IL-2 gene. Increased production of IL-2 further stimulates lymphocytic proliferation (7-9). It was recently suggested that other phospholipids may play a role in the cellular activation process (10). Thus, phospholipase C may catabolize the degradation of PC to diacylglycerol and phosphocholine (11), phospholipase D may cleave PC to phosphatic acid and choline (12, 13), and phospholipases A2 and corresponding lysophospholipases may increase the release of the fatty acid chains (including arachidonic acid), Lyso-PC, and GPC (10, 14).

Nuclear magnetic resonance (NMR) spectroscopy is an excellent method for studying cellular metabolism (15, 16). Its principal advantage over conventional biochemical methods is that it enables continuous monitoring of biochemical processes in intact cells and tissues. We have recently found that 31P NMR spectra of TIL, grown in AIM-V medium with IL-2, are different from those of resting peripheral lymphocytes (17). The major differences were strong signals from glycerophosphocholine (GPC) and glycerophosphoethanolamine (GPE) in the TIL, while they were absent in peripheral lymphocytes. These findings point to the role of other phospholipids, besides phosphoinositol, in activation of lymphocytes.

Cells should be perfused during NMR studies, in order to ensure adequate metabolic status. A useful method to entrap animal cells in sodium alginate capsules was recently presented (18). We have adapted this method for studying the metabolic events which follow lymphocyte activation under various environmental conditions. These studies provide data on the bioenergetic status and phospholipid profile during prolonged lymphocyte stimulation and show that striking elevations in the phospholipid degradation products, GPC and GPE, are general characteristics of lymphocyte activation.

31P NMR techniques also offer the benefits of investigating phospholipid pathways in intact cells. Daly et al. (19) have confirmed by NMR studies of perfused breast cancer cells, that phosphocholine cytidylyltransferase (CT) is the rate-limiting enzyme of PC synthesis in these cells (20-22). 31P NMR signals of phosphocholine in lymphocytes are very low (17, 18, 23), and we present studies on their PC pathways, monitoring the effects of perfusion with choline and CT inhibitor.

MATERIALS AND METHODS

Lymphocyte growth media, used also in perfusion experiments, included AIM-V, RPMI-1640, and IMEM, and were obtained from Gibco Laboratories (Grand Island, NY). All chemicals and glucose and lactate reagents were purchased from Sigma, unless otherwise specified.
Cells—Peripheral blood mononuclear cells were collected through the apheresis technique from healthy volunteers and were separated and concentrated with Ficoll-Pacque (Pharmacia LKB Biotechnology Inc.). The cells were washed twice with growth medium, and contaminating platelets were removed. More than 90% of the recovered cells were lymphocytes (24, 25). Viability was routinely checked with trypan blue staining. TIL were obtained from patients with malignant melanoma, and cells were isolated and expanded as was previously reported (26). The TIL used in these experiments were the surplus of large TIL cultures, used for clinical trials in the Surgical Branch, NCI.

Encapsulation of Lymphocytes in Alginate and Perfusion System—The encapsulation was performed under sterile conditions. 1 ml of lymphocytes pellet (1.0 ± 0.2 × 10⁷ cells) was thoroughly mixed with an equal volume of autoclaved 2.5% (w/w) sodium alginate (low viscosity) in PBS. The mixture was placed in a 1-ml syringe and was manually extruded, under minimal pressure, through a 25-gauge needle, on the surface of 0.1 m calcium chloride solution. The small drops (approximate diameter = 1 mm) gelled and were washed three times in growth medium. The capsules were isolated by decantation, transferred to a 10-mm NMR tube, and perfusion was immediately initiated. Average length of the procedure was 15–20 min, and the period in the CaCl₂ was kept below 5 min.

A simple perfusion apparatus, designed previously for perfusion of cells embedded in agarose threads (27), was used. The alginate capsules were concentrated without compression at the bottom of the tube by an insert with inlet and outlet tubings (0.5-mm inner diameter). The perfusion solution flowed from the opening of the inlet placed near the tube bottom, through the packed alginate capsules, and the outflow was directed to openings in the insert and then to the outflow tubing. A peristaltic pump (Pharmacia P-3) maintained a constant perfusion of 0.5 ml/min at 37 °C. Control spectra were recorded over a 60-90 min period, to ensure stability of metabolite signals. Each series of measurements was repeated at least three times.

Extraction Procedure—TIL and peripheral lymphocytes were washed twice in saline, and the cell pellets (3-4 ml, 2.8–4.6 × 10⁶ cells) were treated with 5 volumes of 0.6 N cold perchloric acid, followed by sonication on ice for 5 min. The mixture was centrifuged for 15 min at 3000 × g, and the supernatant was neutralized by KOH (2 N) and centrifuged to remove KClO₄ precipitate. The extract was passed through a Chelex-100 column (Bio-Rad) and was dried by lyophilization. The extracts were stored at -70 °C to prevent hydrolysis.

Magnetic Resonance Spectroscopy and Data Analysis—31P MRS spectra were recorded on a Varian XL-400 at 162 MHz and were analyzed on a Varian ADS 4000 data station. In intact lymphocyte studies, 3-s repetition time and 60° flip angle were used, and 400 transients were accumulated in each spectrum with 8K data points. Line broadening of 10 Hz was applied. NMR spectra of extracts were recorded with 10-s repetition time, 60° flip angle, 2400 transients, and 4K data points at 4 °C with deuterium lock. Signals were assigned according to previously published data (16–18, 23), and spectral changes in TIL were defined in the extracts by pH titration and adding known compounds. 31P chemical shifts were determined by standardizing GPC to 0.48 ppm, and, when GPC was absent, by standardizing β-ATP to -18.7 ppm.

Both peak heights and peak integrals were measured; since PCho and PEth are only 0.5 ppm apart, their individual integrals could not be accurately determined in intact cells. NMR data acquisition and processing were performed with identical parameters throughout all experiments, and measurements with external reference (methyl-phosphonic acid) showed that quantitative NMR results were thus reproducible. NMR data were normalized according to the protein content, which was measured at the end of each experiment.

Protein, Glucose, and Lactate Determinations—Protein content was measured after the cells were sonicated (Heat Systems, Ultrasonic, Inc.) on ice for 5 min by the bicinchoninic acid assay (Pierce Chemical Co.). Glucose and lactate concentrations were measured in perchlorate treated samples. Glucose was measured by the hexokinase enzymatic assay, utilizing the coupled enzyme reaction catalyzed by hexokinase and glucose-6-phosphate dehydrogenase, and measuring the product, NADH, at 340 nm. Lactate levels were determined utilizing the formation of NAD (and pyruvate) from lactate in the presence of excess NAD. The reaction is catalyzed by lactate dehydrogenase, and the lactate concentrations were calculated from the absorbance at 340 nm. Absorbance measurements were performed with a Shimadzu UV-160 spectrophotometer.

In order to determine the metabolic stability of perfused lymphocytes, control perfusions were maintained for up to 48 h at 37 °C, and there were no spectral changes when the lymphocytes were perfused with growth medium (Fig. 1A). The capsules remained intact, and there were no lymphocytes in the effluent. We found a gradual decrease in ATP signal while perfusing with phosphate-free medium, as was previously suggested (18). Therefore, phosphate-containing growth medium is preferred for prolonged experiments, although intracellular inorganic phosphorus cannot be accurately monitored. The high phosphorus (5.6 mM) RPMI 1640 medium was unsuitable, since the huge extracellular P signal dominated the spectra. Therefore, all perfusion experiments were performed with IMEM (P = 1.1 mM), containing physiologic glucose levels (4 mM) and 10% FCS.

Prior to perfusing lymphocytes with IL-2, it was mandatory to establish that it could penetrate the gel and reach the cells. Since it is difficult to measure diffusion into the alginate support, it was mandatory to determine the metabolic stability of perfused lymphocytes by 31P NMR. Lymphocytes were cast in alginate capsules, perfused with IMEM (4 mM glucose) at 0.8 ml/min and 37 °C, and NMR spectra were continuously recorded. A, control perfusion. The spectra were stable with control perfusions for up to 48 h; B, after 24 h of IL-2 (10⁴ units/ml) perfusion; C, spectrum of perfused TIL, which had been grown for 4 weeks in AIM-V medium and IL-2. Peak assignments: 1, PEth; 2, PCho; 3, P; 4, GPE; 5, GPC; 6, γ-ATP; 7, α-ATP; 8, NAD and DPDE; 9, β-ATP. For acquisition parameters see "Materials and Methods."
capsules, we performed a reverse experiment, i.e. elution from them, since the rates of diffusion in both directions should be the same. The experiment was performed with 14C-labeled egg albumin, and 97% of it was eluted from the alginate capsules within 90 min. Given the difference in the molecular weight between IL-2 (18,000) and albumin (45,000) and the length of the perfusion experiments, it can be concluded that IL-2 experiences no hindrance for access to the cells.

The 31P NMR spectrum of peripheral lymphocytes is characterized by high PEth peak, very low PCho, and no PDE and DPDE signals (Fig. 1A). The main change induced by IL-2 perfusion (human recombinant, Cetus Corporation, Emeryville, CA; 10³ units/ml) was the appearance of the PDE signals (Fig. 1B); however, these signals could be detected only after 12 h of perfusion (Fig. 2). Initially there was a slight increase in ATP levels (11%), which leveled off at about 10 h, but the differences were not significant (p > 0.05 as determined by Student's t test). There were no changes in PME levels. TIL, which were grown for 4 weeks in IL-2, exhibited striking elevation of PDE signals (Fig. 1C). In order to obtain unequivocal assignments of these signals, perchlorate extracts of TIL were studied by 31P NMR (Fig. 3A). By using pH titration curves, and adding the genuine compounds to the extract solution, it was demonstrated that these signals are indeed GPE and GPC. TIL spectra are also characterized by poorly resolved signals at the DPDE region, which are absent in peripheral lymphocytes (Fig. 3B). Several compounds resonate in this region, mainly dinucleotides (NAD and NADP), nucleoside diphospho-sugars (28), and also cytidinediphosphocholine, and we could not assign these peaks.

Extracts of lymphocytes also provided quantitative comparisons between TIL and peripheral lymphocytes regarding the concentrations of these metabolites (Table I). Perfusion experiments were also used for simultaneous monitoring of 31P NMR and glucose metabolism in lymphocytes activated by IL-2 (Table II). As expected, IL-2 induced a significant increase in glucose consumption (p < 0.01); however, under these conditions, lactate production did not follow this elevation and even decreased.

The 31P NMR spectral changes were not unique to IL-2 stimulation; perfusion of lymphocytes with the phorbol ester, TPA (M₀ = f16), caused a similar appearance of PDE signals (Fig. 4). GPE and GPC gradually increased during TPA perfusion, and similar to IL-2 perfusion, are late features of lymphocyte activation. TPA perfusion was followed also by an increase in the phosphocholine peak (Fig. 4, B and C) that was not affected by IL-2.

Since the PDE signals were initially found to be most...
TABLE I
Metabolite concentrations in extracts of lymphocytes

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Peripheral lymphocytes</th>
<th>TIL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>µmol/10^6 cells</td>
<td></td>
</tr>
<tr>
<td>PRthb</td>
<td>7.1 ± 0.6</td>
<td>7.3 ± 0.8</td>
</tr>
<tr>
<td>β-ATPb</td>
<td>4.2 ± 0.5</td>
<td>4.7 ± 0.4</td>
</tr>
<tr>
<td>α-ADPb</td>
<td>1.6 ± 0.3</td>
<td>1.1 ± 0.3</td>
</tr>
<tr>
<td>PCho</td>
<td>0.6 ± 0.3</td>
<td>0.3 ± 0.2</td>
</tr>
<tr>
<td>GPE</td>
<td>nd</td>
<td>2.7 ± 0.3</td>
</tr>
<tr>
<td>GPC</td>
<td>nd</td>
<td>6.4 ± 0.5</td>
</tr>
<tr>
<td>DPDE</td>
<td>nd</td>
<td>0.9 ± 0.3</td>
</tr>
</tbody>
</table>

*Perchloric acid extracts were dissolved in 2 ml of water with 0.2 mM trisodium trimetaphosphate as an internal standard, and metabolite concentrations were accordingly calculated from their ^31P NMR signal integrals. Means and S.D.s of three measurements are presented.

Differences between peripheral lymphocytes and TIL are of no statistical significance, as determined by a paired, double-tailed Student’s t test.

PCho signals were small in both types of lymphocytes, leading to difficulties in accurate measurements of their integrals. Although PCho was higher in peripheral lymphocytes compared to TIL, this was of no statistical significance (p > 0.5).

GPE, GPC, and DPDE signals in peripheral lymphocytes were not detected.

TABLE II
Effects of IL-2 on glucose consumption and lactate production of perfused lymphocytes

<table>
<thead>
<tr>
<th></th>
<th>Glucose</th>
<th>Lactate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>nmol/min × mg protein</td>
<td></td>
</tr>
<tr>
<td>Control, 2 h</td>
<td>6.4 ± 0.4</td>
<td>12.3 ± 1.1</td>
</tr>
<tr>
<td>Control, 6 h</td>
<td>6.3 ± 0.5</td>
<td>11.9 ± 1.0</td>
</tr>
<tr>
<td>IL-2, 2 h</td>
<td>8.7 ± 0.8</td>
<td>9.1 ± 1.1</td>
</tr>
<tr>
<td>IL-2, 18 h</td>
<td>9.4 ± 0.7</td>
<td>8.9 ± 0.9</td>
</tr>
</tbody>
</table>

Lymphocytes were cast in alginate capsules and perfused with IMEM containing 4 mM glucose (and no lactate) at 37 °C. ^31P NMR spectra were simultaneously recorded (see Figs. 1 and 2). In these experiments, 10^5 units/ml of IL-2 was added to perfusion solution after 6 h of control perfusions.

Rates of glucose consumption and lactate production (nmol/min) were calculated by the differences in their concentrations (nm) between the perfusion solution and the effluent, and the perfusion rate (0.8 ml/min). Results were normalized to protein content which was measured at the end of each experiment. Three series of experiments were performed, and in each experiment three samples were taken for all measurements. Thus, the results are means and S.D.s of nine measurements.

pronounced in TIL, it was imperative to check whether this is an inherent metabolic characteristic to these cells. NMR studies require large number of cells; therefore, "early" cultures of TIL could not be investigated. Instead, peripheral lymphocytes cultured with IL-2 for 3 to 21 days were studied (Fig. 5). Fluorescence-activated cell sorter (FACS IV microfluorometer; Becton Dickinson, Mountain View, CA) analyses, performed on days 7–10, showed that 86–96% of the viable cells were T lymphocytes (Leu-2 and Leu-4 antigens). After 3 weeks the ^31P NMR spectra were identical to those of TIL (Figs. 1C and 5C). Moreover, for these experiments the lymphocytes were grown in RPMI medium containing 10% FCS, while TIL were grown in AIM-V medium, which was specifically developed for lymphocytes. Thus, the above noted metabolic changes are independent of the growth media. When the stimulated lymphocytes were washed and recultured without IL-2, PDE signals decreased (Fig. 5D), while the cells remained viable and in a good energetic status. It seems, therefore, that stimulation of lymphocytes is accompanied by prolonged reversible metabolic changes, including

![Figure 4: Effects of the phorbol ester, TPA. ^31P NMR spectra of perfused lymphocytes in alginate capsules. A, after 12 h of TPA (10^-7 M) perfusion; B, 24 h; C, 36 h. Note a gradual increase in the PDE signals, GPE and GPC, and also elevation of the PCho peak. For experimental conditions and peak assignments see legend to Fig 1. For acquisition parameters see "Materials and Methods."](image-url)
A, phate bated with and recultured in RPMI 10% FCS without IL-2. The spectrum was recorded. The spectra were qualitatively evaluated by the $\beta$-ATP signals, + is for high $\beta$-ATP and − is for low or absent $\beta$-ATP. The ratio between PDE and $\beta$-ATP was calculated from the integrals, and served as an indicator to activation changes. In lymphocytes incubated with PHA and nifedipine, and nifedipine alone, ATP signals were very low, and there were no PDE peaks. Means and S.D.s of three series of experiments are presented.

major increase in the phospholipid degradation products, GPC and GPE.

Lympocyte activation requires elevated cytosolic calcium levels (5, 29). In order to examine the mechanism of the NMR observed changes of activated lymphocytes, we incubated peripheral lymphocytes with IL-2 (10$^4$ units/ml) or PHA (leucoagglutinin, 1 mg/ml) and the calcium channel blocker, nifedipine (2 × 10$^{-5}$ M). The results are presented in Table III. The proliferative effect was enhanced in IL-2 compared to PHA, and the PDE signals increase to a greater extent (PDE/$\beta$-ATP integrals ratio 0.89 and 0.47, respectively). It should be noted that studying the effects of PHA in the perfusion system could not be done due to its high molecular weight. Cells treated with IL-2 and nifedipine exhibited high spectra with high ATP levels similar to control cells and markedly increased GPC and GPE. Thus, nifedipine did not prevent the late metabolic changes which are characteristic of IL-2 activation. On the other hand, nifedipine abolished the effects of PHA. The results also demonstrate the requirement of calcium for maintaining ATP levels in resting lymphocytes, which is bypassed when they are stimulated by IL-2.

Phosphatidylcholine synthesis pathways were studied in perfused peripheral lymphocytes. In most cell lines studied by $^{31}$P NMR, the PME region is dominated by PCho (16, 19, 30, 31); however, its concentration in lymphocytes is extremely low (Fig. 6A). This finding led to the assumption that, in contrast to cells with high PCho, choline kinase is the rate limiting enzyme in PC synthesis in lymphocytes. Perfusion with 2 mM choline (normal choline concentration in serum is 0.01-0.02 mM) caused only a minimal increase in the PCho signal (Fig. 6B). Dapsone (4,4'-diaminodiphenyl sulfone) inhibits PC synthesis through inhibition of the enzyme phosphocholine cytidylyltransferase (32, 33). When dapsone was added to the perfusion solution (100 mg/ml), a major increase in the PCho peak was noted (Fig. 6C). The accumulation of PCho only after inhibiting CT by dapsone indicates that CT is not the rate-limiting enzyme of PC synthesis in lymphocytes. These experiments, together with the TPA effects (Fig. 4), point to the regulatory role of choline kinase in this pathway. The time course of phosphocholine changes during choline and dapsone perfusions is presented in Fig. 7; note that the PCho curve in the dapsone experiments is sigmoid in shape indicating some degree of cooperativity in the binding of choline in the presence of dapsone.

---

**Table III**

<table>
<thead>
<tr>
<th>The effects of IL-2, PHA, and nifedipine on peripheral lymphocytes*</th>
<th>$\beta$-ATP</th>
<th>PDE/$\beta$-ATP</th>
<th>Viability</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-2</td>
<td>+</td>
<td>0.89 ± 0.10</td>
<td>100 ± 7</td>
</tr>
<tr>
<td>IL-2 + nifedipine</td>
<td>+</td>
<td>0.73 ± 0.11</td>
<td>76 ± 8</td>
</tr>
<tr>
<td>PHA</td>
<td>+</td>
<td>0.47 ± 0.09</td>
<td>72 ± 7</td>
</tr>
<tr>
<td>PHA + nifedipine</td>
<td>−</td>
<td></td>
<td>53 ± 8</td>
</tr>
<tr>
<td>Nifedipine</td>
<td>−</td>
<td></td>
<td>54 ± 6</td>
</tr>
</tbody>
</table>

*2 × 10$^6$/ml lymphocytes were incubated in RPMI 10% FCS. IL-2 (10$^4$ units/ml), PHA (1 µg/ml), and nifedipine (2 × 10$^{-5}$ M) were added as indicated. Viable cells were counted (with trypan blue staining in a hemocytometer) after 7 days, and the number of viable cells in IL-2 was normalized to 100%. The lymphocytes were then cast into alginate capsules and perfused, and $^{31}$P NMR spectra were recorded. The spectra were qualitatively evaluated by the $\beta$-ATP signals, + is for high $\beta$-ATP and − is for low or absent $\beta$-ATP. The ratio between PDE and $\beta$-ATP was calculated from the integrals, and served as an indicator to activation changes. In lymphocytes incubated with PHA and nifedipine, and nifedipine alone, ATP signals were very low, and there were no PDE peaks. Means and S.D.s of three series of experiments are presented.
Lymphocyte Metabolism $^{31}$P NMR

**DISCUSSION**

$^{31}$P NMR studies provided data on the metabolic changes during activation of lymphocytes in a noninvasive manner. The main result was the finding that activation of resting lymphocytes involves prolonged changes in PC and PE pathways and is characterized by accumulation of their degradation products, GPC and GPE. These $^{31}$P NMR features were common to activation by IL-2, phorbol ester, and PHA. PDEs became the most abundant mobile phosphorus metabolites in lymphocytes activated for 3 weeks and longer. Changes in lipid metabolism during lymphocyte activation were also found in $^1$H NMR studies (34); concanavalin A induced a strong lipid signal.

GPC and GPE are intermediates in the catabolism of PL (35); therefore, their accumulation represents acceleration of PL turnover. Inhibition of phosphodiesterases could also contribute to this phenomenon. Indeed, ethanolamine is a known inhibitor of these enzymes (36) and is present in AIM-V, which was the medium used to expand the TIL cultures. However, we found identical changes using IMEM and RPMI media, which do not contain ethanolamine.

Phospholipase A$_2$ has an important role in the stimulation processes of lymphocytes (10, 37–39). These studies focused on the release and transfer of arachidonic acid, which is further metabolized to prostaglandins. Quite independently, it was shown that Lyso-PL were active as immunomodulators (40), and thus may contribute to the immunological function of activated lymphocytes. GPC and GPE are produced in the next step of catabolism from the corresponding Lyso-PL. GPC was found to have a regulatory role in cellular differentiation (41–43). It was hypothesized that it modulates membranous PL composition and alters membrane fluidity (41). An increase in the fluidity of lymphocyte membranes following activation was previously reported (37, 44, 45). Our results may provide the mechanism of this change. Further studies on the correlation between lymphocyte cytotoxicity and PDEs would determine whether GPC and GPE could serve as quantitative markers of activation in intact lymphocytes.

Enhanced consumption of glucose upon stimulation of lymphocytes was previously noted (46–49) and was attributed to increased energy requirements. This phenomenon was common to activation with TPA (46), PHA (47, 48), and IL-2 (49). Acceleration of glycolysis enzymes (48) and elevation of glycolysis products following conversion from partial aerobic to complete anaerobic glucose utilization were reported (49). However, other investigators found no changes (48), and even elevated activity (47), in the trichloroacetic acid cycle, following stimulation with PHA.

In agreement with these data, we found that IL-2 induced a marked increase in glucose consumption. However, IL-2 induced a reduction in lactate production in our experiments, and thus, no enhancement of glycolysis. Considering the large differences in energetic yield between glycolysis and oxidative phosphorylation, it seems unlikely that the lymphocytes would meet their increased energy requirements by completely shifting to the inefficient anaerobic glycolysis, as suggested (49). Our findings could be explained by an increase in glucose...
utilization through the trichloroacetic acid cycle or utilization of breakdown products of glucose for increased DNA and protein synthesis in activated cells. Similar results were obtained when MDA-468 human breast cancer cells were perfused with epididymal growth factor (31).

It should be emphasized that previous studies used cultured lymphocytes, and substrate depletion and effects of waste materials could lead to artifacts in investigating glucose metabolism. Our system of perfusion, with fresh medium and stable glucose concentrations, offers an advantageous physiological approach and the benefits of simultaneous NMR monitoring of the energetic status. While IL-2 induced major changes in glucose consumption, there were no significant alterations in ATP levels. It is apparent that even under variable metabolic conditions, and during stimulation, balanced levels of ATP are maintained (see also Table I).

The role of calcium in late metabolic events of lymphocyte activation and differences in this regard between lectins (PHA) and IL-2 were investigated using nifedipine. Calcium channel blocking drugs inhibited activation by TPA (50), PHA (51), and IL-2 (52). Our results are in agreement with Rosa-Bono et al. (51) who found that nifedipine inhibits activation by PHA but not by IL-2. Although PDE accumulation was common to both agents, the mechanism is different; PHA activation is coupled to calcium mobilization, while IL-2 bypasses this step.

Vance and Pelech have demonstrated, in a series of fundamental studies, that in many mammalian cells, CT is the rate limiting enzyme of PC synthesis in lymphocytes. Vance and Pelech have demonstrated, in a series of fundamental studies, that in many mammalian cells, CT is the rate limiting enzyme of PC synthesis in lymphocytes. CT was initially demonstrated as the rate-limiting enzyme in the PHLa cells (20–22), and previous NMR studies showed that these cells are characterized by a strong PCho signal (30). However, in lymphocytes, PCho is minimal. TPA was also reported to stimulate PL synthesis by activating CT (21). We found that perfusion with TPA induced an earlier step in the PC pathway and that the PCho signal increased. A similar increase was noted after dapsone perfusion, meaning that under normal conditions, PCho is not accumulated but rather immediately converted to cytidinediphosphocholine. Taken together, these results indicate that choline kinase is the key regulatory enzyme of PC synthesis in lymphocytes.

Acknowledgments—We are very grateful to Dr. K. Shankar-Narayanan, Huntington Medical Research Institute, Pasadena, CA, and Dr. P. Barker, Johns Hopkins Hospital, Baltimore, MD, for their very helpful advice. We would also like to thank Dr. Abeorsold, Surgical Branch, NCI, Bethesda, MD, for providing tumor infiltrating lymphocytes.