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Immunology

Influence of activating stimulus on functional phenotype: Interleukin 2 mRNA accumulation differentially induced by ionophore and receptor ligands in subsets of murine T cells

(*in situ* hybridization/RNA probe protection/CD4, CD8/anti-CD3)

KATHLEEN L. MCGUIRE*, JULIA A. YANG, AND ELLEN V. ROTHENBERG

Division of Biology, California Institute of Technology, Pasadena, CA 91125

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ABSTRACT **We have investigated the linkage between CD4/CD8 phenotype and programing for specific responses in primary T-cell populations. *In situ* hybridization has been used to determine the frequency of cells competent to express the interleukin 2 (IL-2) gene after short-term stimulation with various polyclonal activators. The effects of the T-cell receptor ligands Con A and anti-CD3 monoclonal antibody were compared with those of a calcium ionophore that bypasses membrane receptors altogether. Induction with a calcium ionophore and phorbol ester revealed that potential IL-2 producers not only constitute >85% of the cells with a CD4$^+$ "helper/inducer" phenotype but also constitute over half of the cells with a CD8$^+$ "killer/suppressor" phenotype. There is no defect in the ability of these CD8$^+$ cells to accumulate IL-2 transcripts under these conditions. By contrast, in response to phorbol ester and either Con A or anti-CD3, the CD8$^+$ cells show an abortive IL-2 production response with rapid disappearance of IL-2 mRNA. This results in substantially lower yields of IL-2 per cell than is made by CD4$^+$ cells in response to the same stimuli. The extent to which these populations appear to have diverged in function thus depends on the stimulus used to trigger the response. The results suggest that differences in signal transduction or posttranscriptional regulatory mechanisms, rather than effector gene inducibility *per se*, may initially underlie the commitment of CD4$^+$ and CD8$^+$ cells to distinct functional roles.**

Mature T cells are functionally specialized in their responses to recognition of antigen. In general, T-cell lines that express the CD4 cell-surface glycoprotein are "helper" or "amplifier" cells that respond to the recognition of antigen by secreting lymphokines, often including the major T-cell growth factor interleukin 2 (IL-2) (1, 2). Cells that express CD8 include most killer T cells and show little helper activity (3–6). This suggests that the constitutive CD4/CD8 phenotypes of T cells are correlated with the inducibility of different, limited sets of functional response genes. Although the correlation is frequently observed in memory T cells and T-cell lines, it is not known how it is established during T-cell differentiation. When fresh CD8$^+$ cells are activated and cloned, ≤10% of the clones respond to stimulation by expression of IL-2 (5, 6). This would suggest an intrinsic difference between CD8$^+$ cells and CD4$^+$ cells in programing for gene expression. However, in a variety of other studies, CD8$^+$ cells appear to make IL-2 as well as CD4$^+$ cells (7–11). In the work reported here, we used *in situ* hybridization and quantitative probe protection analyses to measure the accumulation of IL-2 mRNA in individual splenic T cells responding for the first time to different stimuli. We demonstrate that the majority of CD4$^+$ and ≈50% of CD8$^+$ splenocytes are competent to accumulate similarly high levels of IL-2 mRNA when treated with calcium ionophore and phorbol ester. However, the two cell types differ markedly in their kinetics of message accumulation when stimulated by means of the T-cell receptor with concanavalin A (Con A) or an anti-CD3 monoclonal antibody (mAb). The results suggest that primary CD4$^+$ and CD8$^+$ cells differ not so much in their intrinsic abilities to initiate IL-2 transcription but by a mechanism that limits IL-2 message accumulation in the CD8$^+$ cells following stimulation by means of the T-cell receptor.

MATERIALS AND METHODS

Animals. C57BL/6 or C57BL/6-Tla^a mice were bred in our facilities and used at 6–8 weeks of age.

Cell Preparation and Culture. Cell suspensions were prepared from spleen, and CD4$^-$ and CD8$^-$ populations were isolated as described (12). Cultures were incubated with or without 0.1 μM A23187 (Sigma) and 17 nM phorbol 12-myristate 13-acetate (PMA). Where indicated, Con A (Pharmacia) at 6 μg/ml was used to replace A23187. The mAb against murine CD3, clone 145-2C11 (13), was kindly provided by Jeffrey Bluestone (University of Chicago, Chicago) via Linda Sherman (Research Institute of Scripps Clinic, La Jolla, CA) and was added to the cells as a 1:10 dilution of hybridoma supernatant. Note that our culture medium contains 10% fetal bovine serum and that under these conditions we (12) and others (14) find IL-2 RNA expression to be restricted to T cells. The amounts of IL-2 mRNA accumulated per cell in these experiments are higher than we previously reported, due, at least in part, to the use of a different lot of fetal calf serum, which supported higher levels of IL-2 production by all T-cell populations tested (data not shown). Enriched T-cell populations were obtained by two rounds of panning using affinity-purified goat anti-mouse IgG (Boehringer Mannheim) as described (15). In our hands, nylon wool purification resulted in less B-cell contamination but with significant and biased losses of T cells (data not shown).

***In Situ* Hybridization.** Fixed splenocytes were hybridized with the "antisense" IL-2 probe as described (12). Grain counts were obtained from at least 500 cells per slide. The number of cells above background with only one grain was extremely variable between experiments and has not been included in the "positive" cell calculation. To analyze the distribution of IL-2 RNA in responding cells, grain counts from ≥1-month exposures were used. Under these conditions, most uninduced cells still show no grains (12) and cells that fail to respond to induction account for most of the

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Abbreviations: IL-2, interleukin 2; mAb, monoclonal antibody; PMA, phorbol 12-myristate 13-acetate; nt, nucleotide.

*Present address: Dana–Farber Cancer Institute, Jimmy Fund Building, 44 Binney Street, Boston, MA 02115.

zero-grain cells in an induced population (n_i^0). The observed percentage of cells in each grain count category in the induced population, n_i, is the percentage of "true" responding cells, n_i(corr), with specific hybridization above background, plus the percentage of residual nonresponding cells, with all grains due to background. Nonresponding cells are assumed to show the same grain distribution as uninduced cells (n_u percent in the corresponding grain count category). Overall, they should constitute a fraction of the induced population, which, at most, is given by the ratio of the percentages of zero-grain cells in the induced and uninduced populations (n_i^0/n_u^0). Thus, for each grain count category, $n_i(\text{corr}) = n_i - n_u(n_i^0/n_u^0)$. Distributions of n_i(corr) are plotted in Fig. 2. For the percentage of positive cells given in Table 1 (column D), the sum of all n_i(corr) for grain count categories ≥2 was subjected to a further slight correction for potential nonspecific sticking of the probe to the induced cells, by subtracting the sum of n_i(corr) obtained after hybridization with a "sense" strand probe. The sense strand probe gave a higher background than the antisense probe (although <10% cells with ≤2 grains) but showed little difference between induced and uninduced samples. We have previously reported the *in situ* hybridization technique to detect levels as low as 10 molecules per cell in our hands (12).

Ribonuclease Probe Protection. Probe protection measurements of IL-2 RNA have been described in detail elsewhere (12). For quantitation, the 400-nucleotide (nt) *Hin*dIII–*Acc* I probe for the 3′ half of the IL-2 mRNA was used as described. The integrity of the RNA was confirmed by electrophoresis in a 50% urea/5% polyacrylamide gel. The average number of molecules per cell was determined by liquid scintillation spectrometry of protected probe retained on DE81 paper by the following equation: pg of RNA (i.e., pg of probe protected) × (1 pmol/1.2 × 10^5 pg) (i.e., molecular weight of probe = 400 nt × 300 pg/pmol of base) × (1/cell equivalents) × (1 pmol/10^{-12} mol) × (6.03 × 10^{23} molecules per mol). The sensitivity of this technique is <0.1 copy per cell in our hands (12), and no IL-2 mRNA is ever detected in uninduced samples. To map the 5′ end of the message as shown in Fig. 2, a 5′ probe was derived from a genomic 3.0-kilobase (kb) *Eco*RI–*Hin*cII fragment from the 5′ end of the IL-2 gene. This was cloned into pGEM-2, cleaved with *Acc* I, and transcribed to give the 220-nt antisense cRNA probe shown in Fig. 2. Riboprobe control template size markers (Promega Biotec, Madison, WI) were used to determine fragment sizes after electrophoresis. To determine the poly(A) sites used, the 0.8-kb genomic *Eco*RI fragment including the fourth exon of the IL-2 gene (16) was subcloned into pSP65 (T. J. Novak and E.V.R., unpublished results), digested with *Rsa* I, and transcribed with SP6 polymerase. The resulting 456-nt antisense transcript was used as a 3′ probe (data not shown).

RESULTS

We previously demonstrated that mature splenic T cells accumulate large amounts of IL-2 mRNA when stimulated for 24 hr in the presence of the calcium ionophore A23187 and PMA (12). The induction of IL-2 RNA expression in response to these stimuli is not influenced by the presence of accessory cells and is independent of antigenic stimulation (12, 17). Production of IL-2 mRNA is decreased by >95% when all $CD4^+$ and $CD8^+$ T cells are eliminated from the splenocyte population (12). Therefore, even with stimuli that do not require a functional T-cell receptor, IL-2 gene inducibility is a T-cell-specific response under these conditions. As shown in column D of Table 1, after 24 hr of treatment with A23187 and PMA, an average of 20% of surviving spleen cells contain cytoplasmic IL-2 mRNA detectable by *in situ* hybridization analysis. The population of T cells in these splenocyte samples was initially 25–30% and usually declined slightly during culture, suggesting that the large majority of surviving T cells in unseparated splenic cell preparations can activate the IL-2 gene under these stimulation conditions. This interpretation was supported by two additional experiments analyzing responses of enriched splenic T cells. A population with 47% Thy-1^+ cells (27% $CD4^+$, 16% $CD8^+$) at the start of culture yielded 37% with IL-2 RNA and a population with 78% Thy-1^+ cells (42% $CD4^+$, 27% $CD8^+$) responded to stimulation by IL-2 RNA expression in 47%. Quantitative RNase probe protection analysis demonstrated that splenic IL-2-producing cells accumulate, on average, about 650 IL-2 transcripts per positive cell.

To determine whether both $CD4^+$ and $CD8^+$ cells participated in this response, we used mAbs against CD8 and CD4 to mediate complement elimination of mature splenic T cells bearing these cell-surface markers. Over 90% of the susceptible population was eliminated when cells were subjected to two rounds of elimination with the appropriate antibody. This treatment routinely allows recovery of ≈85% of the expected number of total spleen cells in our hands. Additionally, after correcting for viability and total cell recovery, we calculate that >80% of the input spleen cells scoring as IL-2 producers could be recovered in one or the other eliminated population.

Table 1. IL-2 mRNA accumulation by splenic T cells

Spleen	A Viability after culture, % input	B % T cells in initial sample*	C % T cells after culture†	D % positive cells for IL-2 mRNA‡	E Average no. of IL-2 RNA molecules per viable cell§	F Average no. of IL-2 RNA molecules per positive cell¶	G IL-2, units/ml‖
Total	54 ± 4	26 ± 2	20–23	20 ± 4 (3)**	133 ± 33 (5)	665	830
$CD8^-$	61 ± 3	22 ± 0.5	13–15	12.6 ± 2 (3)	100 ± 28	794	670
$CD4^-$	61 ± 1	10.5 ± 0.5	10–13	6.8 ± 0.7	33 ± 3	485	290

All values are the average of two experiments with 24-hr stimulations by A23187 and PMA, except where noted in parentheses. Standard deviations are given.

*As determined by flow cytometry using the rat mAbs 3.155 (anti-CD8) and GK1.5 (anti-CD4) (18) and fluorescein-conjugated anti-rat κ-chain mAb MAR18.5 (Becton Dickinson) on an Ortho system 50 H cytofluorograph with a 5-W argon laser.

†Determined by staining with anti-Thy-1 antibody T24/31.7 (a gift of R. Hyman, Salk Institute, La Jolla, CA) using propidium iodide to exclude dead cells (R. J. Hill and E.V.R., unpublished results). CD4 and CD8 antigens modulate after stimulation.

‡Determined by *in situ* hybridization analysis from 4-month exposures.

§Determined by quantitative RNase probe protection analysis. Quantitation is done by using a ^{32}P-labeled cRNA transcript (12) identical to that used for *in situ* hybridization, which exhibits a lower background than seen in Fig. 1. Values given were derived from two or more independent RNA preparations as indicated.

¶Values in column E divided by those in column D.

‖Units of IL-2 per ml produced in 24 hr by 10^6 cells per ml of culture.

**Two previously reported *in situ* hybridization experiments yielded 17% and 24% of total spleen positive for IL-2 mRNA at 4 weeks exposure (12), which included all cells with ≥1 grain above background.

The levels of IL-2 RNA accumulated by the eliminated populations similarly account for 85% of the IL-2 RNA expressed in the unfractionated spleen cells. Therefore, complement treatment does not appear to interfere with the ability of the surviving cells to express the IL-2 gene.

Quantitative RNase protection analysis (Fig. 1 *A* and *B*) demonstrated that strikingly similar amounts of IL-2 RNA could be induced in eliminated splenocyte populations, whether the cells depleted were $CD8^+$ or $CD4^+$ (compare 4^- and 8^- in lanes marked A23187 + PMA). Table 1 (column E) shows that populations from which all $CD4^+$ cells had been removed accumulated only three times less IL-2 RNA than populations from which only $CD8^+$ cells were removed. As we have previously noted (12), removal of $CD4^+$ and $CD8^+$ cells resulted in >95% loss of inducible IL-2 RNA expression under these conditions. Assuming that B cells and nonlymphoid cells survive equally in both eliminated populations, this implies that a significant fraction of the IL-2 RNA inducible in total spleen is present in $CD8^+$ cells. Indeed, both induced populations included significant fractions of cells with IL-2 transcripts detectable by *in situ* hybridization (Table 1, column D). In at least five experiments with eliminated or unfractionated populations, we have noted that $CD8^+$ cells are slightly enriched and $CD4^+$ cells are slightly depleted following stimulation (Table 1, column C, and unpublished results). Hence, the 12.6% positive cells in the $CD8^-$ population presumably reflect responses from among the 13–15% $CD4^+$ cells remaining, and the 6.8% positive cells in the $CD4^-$ population were drawn from the 10–13% $CD8^+$ cells (Table 1, compare columns C and D). This suggests that at least 80% of $CD4^+$ cells and about 50% of $CD8^+$ cells can express IL-2 RNA, as discussed further below.

The accumulation of IL-2 RNA in response to A23187 and PMA was similar in individual $CD4^+$ and $CD8^+$ cells. As demonstrated by the representative *in situ* hybridization analysis shown in Fig. 2, the grain distributions for IL-2 producers within the $CD4^-$ and $CD8^-$ depleted populations were hardly distinguishable, except in the overall percentage of cells involved. This result agrees with calculations from the quantitative RNase protection analysis, which implied that $CD8^+$ IL-2 producers accumulate high levels of IL-2 RNA (≈500 molecules per cell), although somewhat lower, on average, than individual $CD4^+$ IL-2 producers (≈800 molecules per cell; Table 1, column F). As shown in column G of Table 1, IL-2 protein was secreted by all populations in relative amounts consistent with the amount of IL-2 RNA present at 24 hr (compare columns D and F). RNase mapping of the IL-2 RNA accumulated by these cells demonstrated that all three populations use identical 5′ start and 3′ poly(A) sites (Fig. 1 and data not shown), further indicating that the IL-2 RNA present is in the form of the mature, functional message. Taken together, these measurements indicate that about half of the surviving $CD8^+$ T-cell population can respond to A23187 and PMA by accumulation of *bona fide* IL-2 mRNA to levels comparable to those in $CD4^+$ cells.

To test further whether such a high fraction of $CD8^+$ cells is inducible, we have used two other sets of results to calculate the expected frequencies of potential IL-2 producers, x and y, in the $CD8^+$ and $CD4^+$ populations, respectively. One set of results is that for total spleen (Table 1) in which about 23% of surviving cells after stimulation were T cells. The relative survival rates of $CD4^+$ and $CD8^+$ cells (column C) imply that 10% were $CD8^+$ and 13% were $CD4^+$. The second set of results is from the analysis of enriched T cells described above, in which 42% of input cells were $CD4^+$

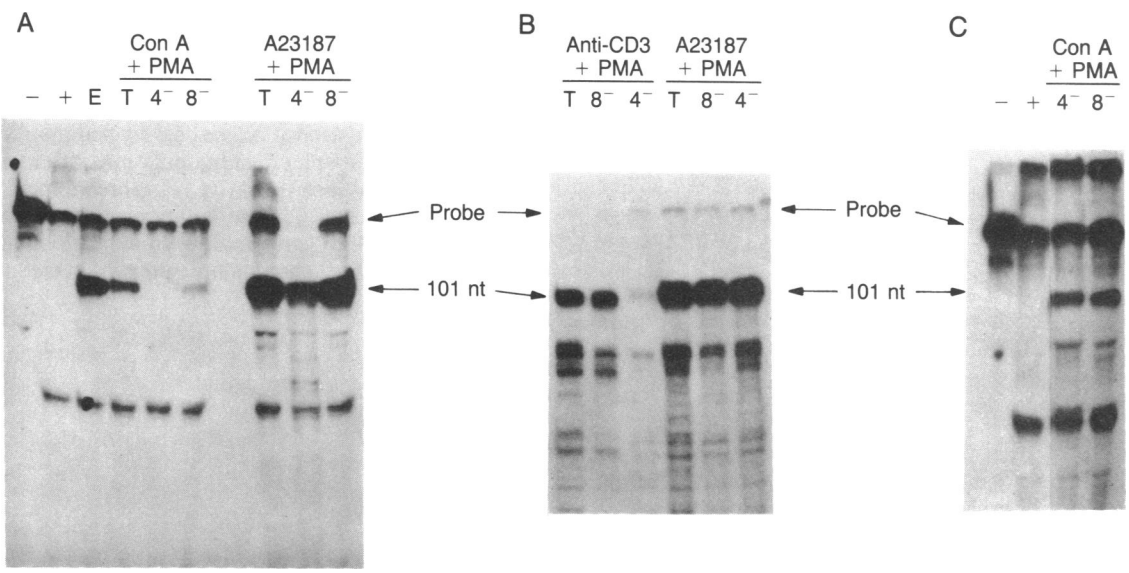

FIG. 1. Quantitation and mapping of 5′ ends of IL-2 mRNA accumulated by splenic T cells in response to various stimuli. (*A*) RNase protection of an *Acc* I–*Hinc*II cRNA probe spanning the IL-2 cap site, using cytoplasmic RNA isolated after 24 hr in culture with A23187 or Con A and PMA. The migration of the fragment protected by RNA with the reported IL-2 cap site is marked as 101 nt. − or +, Probe incubated with 20 μg of yeast tRNA and carried through the analysis as described below without (−) or with (+) RNase A and T1 treatment, respectively. E, 20 μg of RNA isolated from the thymic lymphoma EL4.E1, stimulated for 4 hr with 10 ng of PMA per ml, as a positive control. T, Total spleen; 4^-, $CD4^-$ spleen; 8^-, $CD8^-$ spleen. For all samples stimulated with A23187, 5×10^6 cell equivalents (16–21 μg of RNA) were used, and for the total, $CD4^-$, and $CD8^-$ Con A-stimulated samples, 10^7, 1.3×10^7, and 9×10^6 cell equivalents (20–28 μg of RNA) were used, respectively. The exposure time shown was 2 days. IL-2 RNA levels in these Con A-stimulated samples were measured to be 6 molecules per cell for total spleen, 2 molecules per cell for $CD8^-$ spleen, and 0.2 molecule per cell for $CD4^-$ spleen. (*B*) Total, $CD4^-$, and $CD8^-$ splenocytes were stimulated for 24 hr with PMA and either A23187 or a 1:10 dilution of anti-CD3 hybridoma supernatant, and 1×10^7 cell equivalents of cytoplasmic RNA from each was used to protect the IL-2 probe. The exposure time was 20 hr. In these samples, the 5′ probe was used for quantitation. The levels of IL-2 RNA per cell were calculated to be 207, 173, and 206 molecules per cell for the total, $CD8^-$, and $CD4^-$ cells stimulated with A23187 and PMA, respectively, and 26, 23, and 1 molecule per cell for total, $CD8^-$, and $CD4^-$ cells stimulated with anti-CD3 and PMA, respectively. (*C*) RNase protection as in *A*, using 2×10^7 cell equivalents (30–44 μg) of cytoplasmic RNA isolated from cells stimulated for 6 hr. This film was exposed for 6 days. The levels of IL-2 RNA quantitated in these samples were 10 molecules per cell for total spleen, 5 molecules per cell for $CD8^-$ spleen, and 3 molecules per cell for $CD4^-$ spleen.

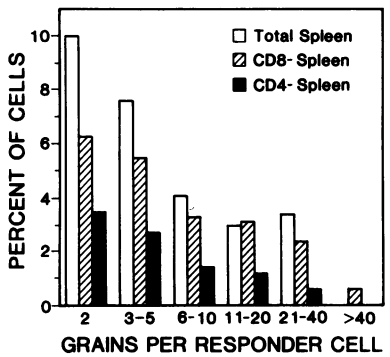

FIG. 2. Distribution of IL-2 mRNA accumulated in different classes of splenic IL-2 producers in response to A23187 and PMA. Total, CD4-eliminated, and CD8-eliminated spleen cells were cultured with or without inducers and probed for IL-2 RNA by *in situ* hybridization. The histograms plot the corrected percentages of cells in induced populations [n_i(corr)] that exhibited the indicated numbers of grains in a representative experiment. Higher grain numbers are associated with higher numbers of transcripts per cell (12). The grain counts for each population, induced and uninduced, were determined from a 4-week exposure (29 days) of a representative experiment, and the corrected histograms were derived by subtracting background values. The similarity between the distributions of IL-2 RNA among producing cells in all three populations was observed in three experiments.

and 27% were CD8$^+$. Here, about 90% of the input cells were viable 24 hr after stimulation, suggesting only minor population changes. At most, if all dying cells were CD4$^+$, the stimulated cells were 35% CD4$^+$ and 30% CD8$^+$. These values can be used to solve for x and y with two independent equations. (*i*) The fraction of cells positive by *in situ* hybridization is

$$\text{Fraction inducible} = (\text{CD4}^+ \text{ poststimulation})y + (\text{CD8}^+ \text{ poststimulation})x. \quad [1]$$

(*ii*) As shown in Table 1, column E, CD4-eliminated spleen cells make one-third as much IL-2 RNA as CD8-eliminated spleen cells (i.e., 33 ± 3 vs. 100 ± 28). Then, if an average CD8$^+$ responder has 500 copies of IL-2 RNA and an average CD4$^+$ responder has 800 copies, it follows that (CD8$^+$ poststimulation)(500x) = 1/3(CD4$^+$ poststimulation)(800y), or

$$(\text{CD8}^+ \text{ poststimulation})x = 8/15\ (\text{CD4}^+ \text{ poststimulation})y. \quad [2]$$

In the total spleen analysis, the average fraction inducible is 0.2 (Table 1, column D). By using our estimates for CD4$^+$ and CD8$^+$ subsets after stimulation, $y \approx 1.0$ and $x = 0.7$, with almost all CD4$^+$ cells and over half of CD8$^+$ cells responding. Similarly, in the experiment with enriched T cells, 0.47 of the cells could express IL-2 RNA. Depending upon whether CD4$^+$ cells are assumed to have died preferentially, values for y range from 0.73 to 0.9 and those for x range from 0.54 to 0.61. These are in good agreement with the previous values and those estimated from CD4- or CD8-eliminated populations (Table 1). Altogether, these calculations indicate the consistency of the *in situ* hybridization analyses and support the competence of at least half of splenic CD8$^+$ cells to make IL-2.

To test whether the mode of triggering influenced responsiveness, we replaced A23187 with either of two polyclonal stimuli that could mimic more physiologic triggering by binding to the T-cell receptor—i.e., the lectin Con A or a mAb against the ε subunit of CD3. In all cases, PMA was added to obviate the requirement for accessory cells. RNase probe protection analysis demonstrated that 24 hr of culture with 6 μg of Con A per ml stimulated spleen cells to express IL-2 mRNA, although at lower levels (by a factor of ≈10–20) than in cultures induced with A23187 (Fig. 1 legend). Consistent with the levels of RNA detected, Con A-stimulated cultures also secrete, on average, fewer units of IL-2 activity per ml (by a factor of 10–30) than A23187-stimulated cultures (data not shown). However, the effects of Con A on CD4$^+$ and CD8$^+$ cells were not the same. As shown in Fig. 1*A*, probe protection analysis revealed that CD4$^-$ splenocytes expressed only about 1/10th as much IL-2 mRNA as CD8$^-$ cells after 24 hr of stimulation with Con A and PMA. They correspondingly secreted lower titers of IL-2 (by a factor of 8–10) than CD8$^-$ splenocytes during the incubation (not shown). The poor IL-2 response of CD8$^+$ cells did not result from an overall inability to respond to Con A and PMA, for CD8$^+$ cells were fully activated to express IL-2 receptors (J.A.Y., data not shown). As shown in Fig. 1*B*, CD8$^+$ cells also expressed less IL-2 mRNA (by a factor of at least 10) than CD4$^+$ cells when stimulated for 24 hr with anti-CD3 and PMA. Thus, although many or most CD8$^+$ cells can accumulate IL-2 mRNA similarly to CD4$^+$ cells when stimulated with a calcium ionophore, they do not appear to do so when stimulated by Con A or by anti-CD3.

The differences in overall accumulation of IL-2 protein are consistent with a prolonged disparity between the levels of translatable IL-2 mRNA in CD4$^+$ and CD8$^+$ cells, but they do not rule out a brief response in the CD8$^+$ cells. This was of concern because time course analysis of IL-2 RNA accumulation in total spleen revealed that the kinetics of the accumulation were different in response to Con A and PMA than for A23187 and PMA (Fig. 3). In response to Con A, an initial peak was seen at 6 hr of stimulation, then a decline and a second peak at 24 hr, instead of the continued increase through 24 hr of stimulation induced by A23187. This is consistent with previous reports of a peak in IL-2 mRNA expression ≈5 hr after induction with mitogen (9, 10, 19). To analyze the initial activation process, we compared the levels of IL-2 mRNA in splenic subpopulations after only 6 hr of stimulation with Con A (Fig. 1*C*). In three experiments with either Con A or anti-CD3 stimulation, we found that CD8$^+$ cells contribute at least half as much accumulated IL-2 mRNA as CD4$^+$ cells at this time point. This result suggests

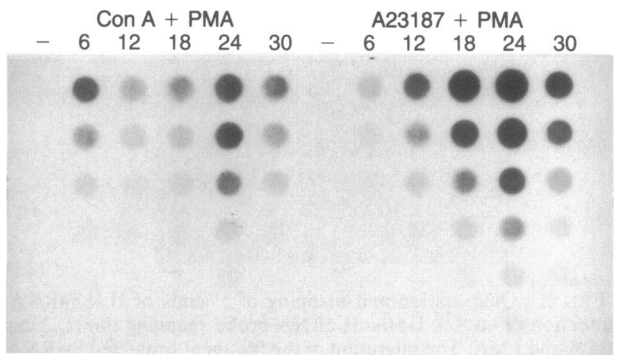

FIG. 3. Time course of IL-2 mRNA production by total spleen cells in response to different stimuli. Cell cultures consisting of 4 × 10^7 cells supplemented with Con A and PMA and 2 × 10^7 cells supplemented with A23187 and PMA were incubated for the indicated times (given in hours). One-half of the resulting cytoplasmic RNA isolated from each culture was blotted in descending 2-fold serial dilutions as described (12). Viable cell equivalents ranged from 13 to 20 × 10^6 cells for Con A and 6.5 to 10 × 10^6 cells for A23187, with the lowest numbers of viable cells in the 6-hr Con A and 24-hr A23187 cultures and the highest numbers in the uninduced controls. The autoradiograph shown is an overnight exposure of the filter hybridized with an IL-2 cDNA probe.

that CD8$^+$ T cells may initially activate the IL-2 gene when stimulated by means of the T-cell receptor for antigen, but that under these conditions, CD8$^+$ and CD4$^+$ cells differentially regulate subsequent IL-2 mRNA accumulation.

DISCUSSION

We have used *in situ* hybridization to measure the competence of individual splenocytes to express the IL-2 gene prior to any antigen-driven clonal expansion. This approach has revealed a strikingly broad phenotypic distribution of cells with intrinsic competence to make IL-2, for many or most CD8$^+$ cells as well as CD4$^+$ cells are fully competent under certain stimulation conditions. A difference between CD4$^+$ and CD8$^+$ IL-2 producers emerged, however, in their responses to different polyclonal activators. Though the calcium ionophore induced sustained IL-2 RNA accumulation in both, the membrane receptor ligands Con A and anti-CD3 provoked a limited IL-2 production response in the CD8$^+$ cells as compared to the CD4$^+$ cells. Thus, many CD8$^+$ splenocytes appear to differ from CD4$^+$ cells not so much in their potential to express the IL-2 gene as in their interpretations of particular activation stimuli.

Previous work (9–11, 20, 21), using bulk culture and limiting dilution analysis, has established that some CD8$^+$ cells have the ability to secrete IL-2, but it did not reveal the prevalence of potential IL-2 producers in the CD8$^+$ population that we observe upon stimulation with calcium ionophore and PMA. Several methodological differences may explain our findings. We have looked within the first 24 hr of stimulation, which may be important for detecting IL-2 production by CD8$^+$ cells (cf. refs. 20 and 21). In particular, *in situ* hybridization analysis allows us to look at the primary T-cell response prior to clonal expansion. Recent studies using the Pgp-1 marker to identify memory cells (22) support the interpretation that most (70%) of our splenic CD8$^+$ cells are indeed virgin T cells. By contrast, A23187 and PMA do not induce IL-2 RNA in many long-term killer cell lines (T. J. Novak and E.V.R., unpublished data), suggesting either that CD8$^+$ cells may lose this capacity after exposure to antigen (20, 21), or that those that proliferate extensively *in vitro* come primarily from the noninducible fraction. In any case, the inducibility of IL-2 in about half of the CD8$^+$ cells after stimulation with calcium ionophore and phorbol ester clearly shows that in virgin CD8$^+$ cells the IL-2 gene need not be locked in an inactive chromatin configuration. The gene itself may always be competent to be expressed in mature primary T cells.

Although there appears to be little difference between CD4$^+$ and CD8$^+$ cells in the potential inducibility of their IL-2 genes by A23187 and PMA, there is a clear difference in their abilities to accumulate IL-2 mRNA following prolonged stimulation with PMA and either Con A or mAb directed against CD3. Similarly, in response to antigen, IL-2 production is apparently limited to certain responding T cells dominated by, though not restricted to, cells of the CD4$^+$ phenotype (4–6). Our results from the 6-hr stimulation with T-cell receptor ligands, as well as previous reports in the literature (9, 10), support the hypothesis that transcription may initially be comparable in the two cell types but that RNA accumulation is limited at a later step in CD8$^+$ cells. Such a mechanism might affect the accumulation of IL-2 transcripts in individual cells or decrease the frequency of responding cells. Several laboratories have reported that cycloheximide, which blocks protein elongation, enhances the lectin-induced accumulation of IL-2 mRNA (9, 10, 23) without affecting the transcriptional activity of the gene (24). These data suggest that a protein product may be responsible for limiting IL-2 mRNA accumulation after stimulation with lectin. CD4$^+$ and CD8$^+$ T cells might differentially express this regulatory protein product, with its expression being particularly prominent in CD8$^+$ lymphocytes. The accumulation of high levels of IL-2 mRNA in both subpopulations of mature T cells and the kinetics of that response suggest that ionophore and phorbol ester may bypass this proposed regulatory mechanism altogether. Further work is required to test this possibility directly. However, the results presented here persuasively suggest that the difference between cells committed to "IL-2-producing" and "non-IL-2-producing" lineages may not be attributed either to the chromatin structure of the IL-2 gene or to the initial availability of specific transcription factors for IL-2. Particularly in these primary T-cell populations, signal transduction and/or RNA stabilization mechanisms may play a vital role in regulating a surprisingly plastic set of functional responses.

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