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Cell division regulates the T cell cytokine repertoire, revealing a mechanism underlying immune class regulation

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ABSTRACT **Naive T lymphocytes have the potential to differentiate and produce a range of cytokines crucial for appropriate immune responses. How T lymphocytes vary their cytokine output during differentiation is unknown, although they are clearly influenced by the cytokines already present in the environment. Here we show that the number of divisions taken by the cells after activation is a critical element in T cell differentiation. Our experiments used the dye 5-(and 6-)carboxyfluorescein diacetate, succinimidyl ester to track cells in different divisions after activation by anti-CD3 in the presence of the differentiating cytokine interleukin (IL)-4. The patterns of acquisition or loss of secretion of IL-2, IL-3, IL-4, IL-5, IL-10, and interferon γ all varied markedly with division number. These relationships were consistent regardless of the time-dependent variation in distribution of T cells among divisions. Thus, the observed combination of complex asynchronous T cell growth, overlaying a fixed probability of acquisition or loss of a cytokine at each division can explain why T cell differentiation displays the contradictory features of being both highly stochastic and highly controlled. Furthermore, these data reveal that T cells share a common regulatory strategy with B cells, whereby changes in the class of immune response are linked to the process of clonal expansion.**

The mechanism whereby the immune system determines which cytokines to produce in response to a particular antigen is of great interest because successful immunity depends on making the correct decision. Over the past decade, T cell clones and bulk populations of cells have been broadly classified into at least three classes, designated as Th0, Th1, and Th2, based on cytokine expression (1–4). However, this simple picture becomes far more complex after analysis of single cells isolated from the same population. Such methods reveal many more cytokine combinations per cell, which when correlated with both the type and level of cytokine secreted, demonstrate remarkably random associations (5–7) and argue for a highly stochastic component to T cell differentiation. Further complexity is introduced by the identification of many factors in addition to the cytokine environment (8–11) that affect differentiation, including the type of antigen-presenting cell (APC) (12–15) and the dose and nature of the antigen (16–18).

The influence of so many variables implies that the T cell is capable of sophisticated decision making as a prelude to selecting a particular course of differentiation. However, one variable of great potential importance, the number of divisions T cells achieve after stimulation, has received little attention. With the development of methods for fluorescent cell division tracking by Lyons and Parish (19), the issue has become accessible to analysis at least through the first seven or eight divisions. When applied to B cell differentiation, cell division number was found to be the single most important predictive variable in isotype switching (20, 21). These results suggested a logic to isotype switching where persistence of antigen would act as a feedback to regulate the class of immune response (21). Isotype switching by B cells has obvious parallels with T cell regulation of cytokine repertoires and led us to the hypothesis that division also would play a crucial role in T cell differentiation. The initial results of experiments designed to test this hypothesis are presented here and provide convincing evidence of a link between the type and amount of cytokine produced and the division number of naive T cells.

MATERIALS AND METHODS

Mice. Female B10.BR mice aged 8–12 weeks (Animal Resources Centre, Perth, Australia) were housed at the Centenary Institute under specific pathogen-free conditions.

Cell Preparation and Staining. Single-cell suspensions derived from lymph nodes (axillary, brachial, cervical, inguinal, and para-aortic) were enriched for T cells by complement lysis of $B220^+$ (RA3.3A1) (22), HSA^{hi} (J11d) (23), $CD8^+$ (31M), and $CD25^+$ (7D4) (24) cells. Viable cells were labeled with CD62L-phycoerythrin (MEL-14, PharMingen), CD44-fluorescein isothiocyanate (IM7) (25) and biotinylated antibodies against B220 (RA3.6B2) (26), $I\text{-}E^k$ (14-4-4S) (27), CD8 (53–6.7) (28) CD25 (PC61) (29) followed by streptavidin tricolor (Caltag, Burlingame, CA). Sort gates on a FACStar^{Plus} (Becton Dickinson) were set to exclude any tricolor positive cells. Positive sorting was restricted to small (by light scatter profiles), $CD62L^{hi}$, $CD44^{lo}$ cells. After sorting cells were typically >98% $CD62L^{hi}$, $CD44^{lo}$, $CD4^+$.

Primary Stimulation. Sorted cells were stimulated at $5–10 \times 10^5$ cells/ml with plate-bound anti-CD3 (10 μg/ml, 145–2C11) (30), interleukin (IL)-2 (100 units/ml), and IL-4 (100 units/ml) in the presence of anti-interferon (IFN)-γ antibody (AN18) (31) at 10 μg/ml. Controls cultured without IL-4 included anti-IL-4 antibody (11B11) (32) at 10 μg/ml. Culture medium was RPMI medium 1640 with L-glutamine (2 mM), heat-inactivated fetal calf serum (10%), 2-mercaptoethanol (5×10^{-5} M), and penicillin (100 μg/ml) and streptomycin (100 units/ml).

Analysis of Cell Division. Cells were labeled with 5-(and-6)-carboxyfluorescein diacetate, succinimidyl ester (CFSE, Molecular Probes) according to Lyons and Parish (19). Suspensions of 10^7 cells/ml in 0.1% BSA/PBS were incubated with CFSE at a final concentration of 10 μM for 10 min at 37°C, then 10× volume of cold 0.1% BSA/PBS solution was added.

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Abbreviations: CFSE, 5-(and-6)-carboxyfluorescein diacetate, succinimidyl ester; BrdU, 5-bromo-2′-deoxyuridine; IL, interleukin; IFN, interferon; APC, antigen-presenting cell.

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5-Bromo-2′-Deoxyuridine (BrdU) Analysis. Cells stimulated with immobilized anti-CD3 (10 μg/ml) at 5×10^5 cells/ml as described above were pulsed with 100 μg/ml of BrdU (Sigma) for the final 7 h or 9 h of culture before harvesting at days 2, 3, 4, or 5. Cells were suspended in PBS with 1% BSA and fixed for 10 min with 1% formaldehyde (Sigma), then diluted with an equal volume of Tween-20 (final 0.1%, ICN) and incubated overnight. Tween-20 (0.01%) and DNase-1 (50 μg/ml, Boehringer-Mannheim) were added at 37°C for 30 min before BrdU was detected with biotinylated anti-BrdU antibody (Caltag) and streptavidin tricolor (Caltag).

Cytokine Secretion by Cells Sorted According to Division Number. The CFSE peaks were sorted by a FACStar or FACStar^{Plus} flow cytometer (Becton Dickinson). Control cultures of CFSE-labeled but unstimulated cells, harvested on the same day, provided a reference for the fluorescence intensity of undivided T cells. Sort gates were selected for viable cells (determined by light scatter profiles) falling within the appropriate CFSE peaks. The purity of these cells was typically ≈80–85%. Sorted cells were restimulated with immobilized anti-CD3 (10 μg/ml) at 10^6 cells/ml culture. After 24 h, cells were harvested for analysis of CFSE profiles, and supernatants were assayed for cytokines by specific sandwich ELISA (IL-4, IL-5, IL-10, and IFN-γ) or bioassay for IL-2 using HT2 cells (33) with 11B11 (5 μg/ml) to block IL-4 and 32DCL cells to assess IL-3 secretion (34). Levels of cytokines in supernatants were evaluated by comparison against standards of known concentration.

Intracellular Cytokine Staining. After primary stimulation, CFSE-labeled cells were restimulated for 6 h with immobilized anti-CD3 (10 μg/ml) at 10^6 cells/ml. Intracellular cytokines were detected by a modified method of Sander *et al.* (35), using saponin (0.5%) as the permeabilizing agent and brefeldin A (10 μg/ml, Sigma) added at 2 h to prevent secretion before staining (36). Cells were fixed with formaldehyde (4%) at room temperature for 20 min followed by incubation with phycoerythrin-conjugated anticytokine antibodies JES6–5H4 (anti-IL-2), MP2–8F8 (anti-IL-3), 11B11 (anti-IL-4), TRFK5 (anti-IL-5) or JES5–16E3 (anti-IL-10) (PharMingen) or XMG (anti-IFN-γ, Caltag). Nonspecific uptake and retention of antibodies were controlled by blocking control samples with nonconjugated preparations of cytokine-specific antibodies or with excess cytokine in the case of IL-2. The Th2 cell line D10G4.1 (37) was used as a positive control for the stimulation and staining procedures.

RESULTS

IL-4 Is a Differentiation Factor for Naive T Cells. To investigate our hypothesis that division number regulates the T cell cytokine repertoire we chose a culture system that minimized the influence of variables that might obscure the effects of division. Previous studies have demonstrated that APC-independent cultures of anti-CD3-stimulated naive T cells proliferate but do not produce IL-2 and IL-4 on restimulation unless the differentiation factor IL-4 also is included (8, 11, 38–40). These results were confirmed by culturing naive CD4$^+$ T cells (CD62L^{hi}, CD44^{lo}, CD25$^-$) (41–44) with anti-CD3 and IL-2 with or without IL-4. After 4 days, cells were harvested, washed, and restimulated for assessment of cytokine production. The cytokines IL-4, IL-2, and IFN-γ were found at high levels only from cells derived from primary cultures stimulated in the presence of IL-4 (Fig. 1*A*). The production of IFN-γ was surprising as IL-4 is known to inhibit development of T cells secreting this cytokine upon restimulation (8, 11, 38). However, Lederer *et al.* (40) have noted that the APC plays an active role in this inhibition as T cells cultured in IL-4 secreted high levels of IFN-γ when restimulated with anti-CD3 in contrast to antigen presented by APC.

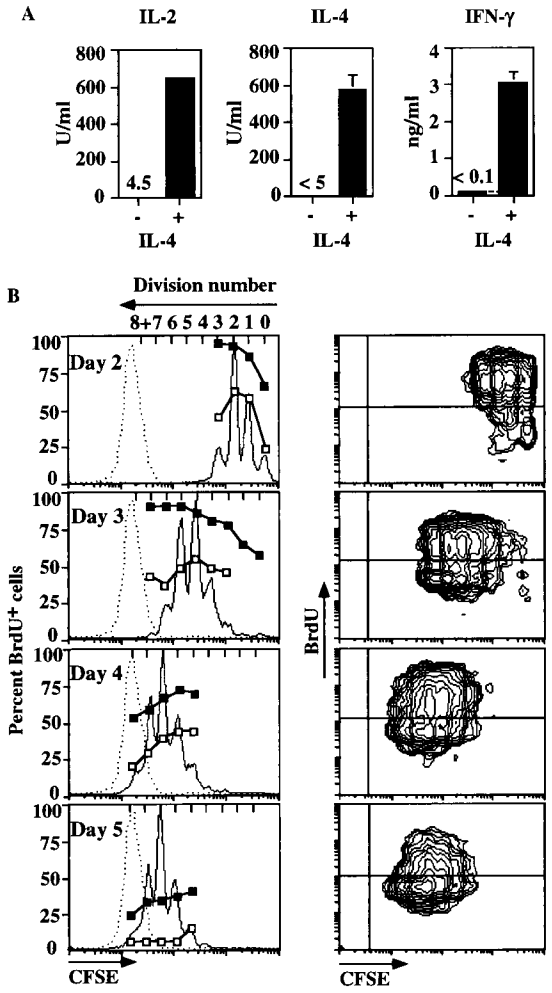

FIG. 1. IL-4 production and asynchronous division of stimulated T cells. (*A*) T cells were stimulated in primary culture with immobilized anti-CD3 and IL-2 with or without IL-4. After 4 days, activated cells were washed and restimulated with anti-CD3 (10 μg/ml) for 24 h. Supernatants were assayed for IL-2, IL-4, and IFN-γ. (*B*) CFSE profiles and BrdU labeling of dividing T cells at different times. CFSE-labeled T cells were stimulated with anti-CD3, IL-2, and IL-4 and harvested on days 2–5 as indicated. Histograms of CFSE fluorescence are shown with autofluorescence of nonlabeled cells overlayed as a dotted line (*Left*). (*Right*) BrdU incorporation by CFSE-labeled cells after a 9-h pulse (7 h for day 2). Contours (log density, 70%) are plotted with CFSE as abscissa and BrdU as ordinate. The percent BrdU$^+$ cells in each division at each time is overlayed on the division histogram on the left and shows percent positive after either 1 h (open symbol) or 9 h (closed symbol).

Therefore, by using anti-CD3 with IL-2 and IL-4 the division-dependent differentiation response to a minimal set of signals can be defined.

Naive T Cell Proliferation and Differentiation Can Be Monitored by Using CFSE. Labeling with the fluorescent dye CFSE was used to investigate the role of cell division in the differentiation of T cells in the above cultures (19). Upon division, CFSE is distributed evenly between daughter cells, and the mean fluorescence halves accordingly. In this way, division progression can be analyzed by flow cytometry, with division numbers defined by gates set around CFSE fluorescence peaks (19). The limit of detection is seven or eight division cycles caused by compression of peaks as the CFSE intensity approaches autofluorescent levels (Fig. 1*B*). Thus, divisions beyond seven cycles are indistinguishable and are collectively referred to as divisions 8+.

CFSE-labeled naive T cells stimulated with anti-CD3, IL-2, and IL-4 were analyzed on days 2–5 (Fig. 1*B*). Division patterns

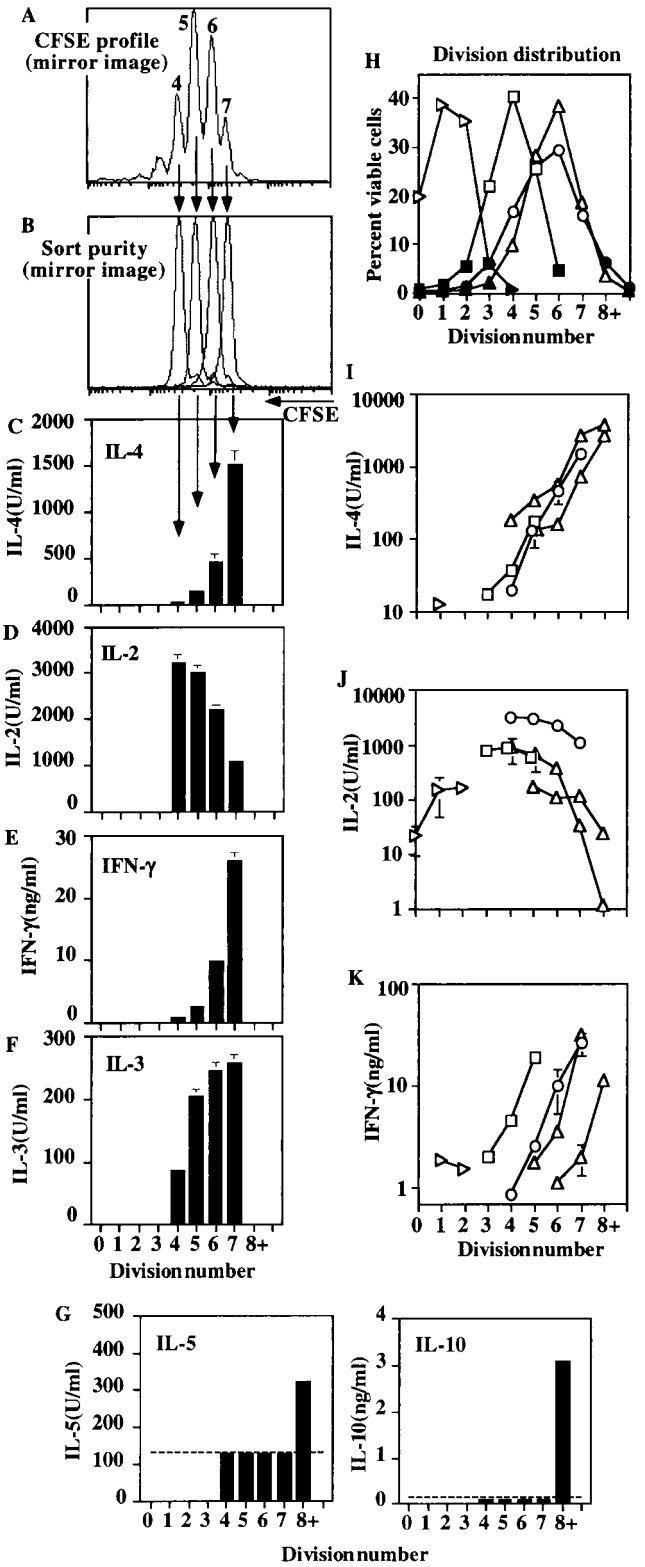

FIG. 2. Division-related changes in secretion of IL-4, IL-2, and IFN-γ. Naive, CD4+ T cells labeled with CFSE were stimulated with anti-CD3, IL-2, and IL-4 after which the asynchronously dividing population was harvested and sorted according to CFSE intensity. Each sorted population was restimulated with anti-CD3 at 10^6 cells/ml for 24 h before harvesting supernatant. (*A*) To align with plots of division number the mirror image of a day 4 CFSE profile from which cells were sorted is presented. The brightest (least divided) cells are at the left. (*B*) Overlay of CFSE histograms showing the cells after sorting into populations of uniform division number. (*C*) IL-4 secreted by cells in divisions 4–7. Similar assays were conducted for (*D*) IL-2, (*E*) IFN-γ, and (*F*) IL-3. Each column represents the mean and standard error of at least three replicates. (*G*) Detection of IL-5 and IL-10 from a representative day 5 culture. (*H–K*) Purified naive, CFSE-labeled T cells were stimulated in primary culture for either 2 (▷), 3 (□), 4 (○), or 5 (△) days (two experiments) followed by cell sorting by division, restimulation with anti-CD3 and harvest of 24-h supernatants. Each point shows the mean and standard error of at least three separate cultures except divisions 8+ (single samples) for each day 5 experiment and day 3 division 3, which is in duplicate. (*H*) The proportion of viable cells found in each division at each time point. Divisions represented by open symbols were sorted for restimulation. Where possible, overlapping division cycles were selected at consecutive time points. Supernatants were analyzed for the presence of (*I*) IL-4, (*J*) IL-2, or (*K*) IFN-γ.

were affected by the starting cell density and the concentration of anti-CD3 (data not shown). Under the chosen conditions, cell division was in progress by day 2 with a group of cells already having reached the third division. By day 3, the population was dominated by dividing cells proceeding as a wave distributed evenly around a mean division number. Proliferation had slowed by day 4 because of media depletion but the cells retained the asynchronous division pattern with little loss of viability over the next 24 h (Fig. 1*B*). Analogous cultures of T cells deprived of IL-4 were slightly slower to initiate division but then progressed at a similar rate with the mean cell division number being reduced by one each day (data not shown). Therefore, the marked differences in cytokine production in the two populations cannot be accounted for by differential rates of growth.

Kinetic Features of Division Asynchrony Revealed with BrdU and CFSE. To investigate the dynamic nature of cell division in each of the division cycles after varying periods of primary culture, stimulated T cells were pulsed with BrdU. This method allowed the proportion of cells that entered S phase of the cell cycle during the pulse to be determined (Fig. 1*B*). The percentage of cells positive for BrdU in each division after two different pulse durations is superimposed on the CFSE histograms in Fig. 1*B*. At day 2, the undivided cells were mitotically active with 50% of these cells being labeled during the longer pulse. By day 3, the small proportion of undivided cells still detectable also labeled with BrdU, indicating that the time to enter the first division was highly variable. Cells in divisions beyond the first displayed relatively consistent BrdU incorporation, reflecting uniform rates of division. Therefore, cells in the later divisions (5 to 8) were not, on average, proliferating more rapidly than those in divisions 2–4 but had entered the first division cycle at an earlier time.

Cytokine Secretion Varies with Division Number. Division-related development of cytokine-secreting capacity was studied first by using cell sorting to separate T cells according to division cycle number (Fig. 2 *A* and *B*). Sorted populations were restimulated at equal cell densities with immobilized anti-CD3 and 24-h supernatants harvested for quantitation of cytokines. Fig. 2*C* illustrates the levels of IL-4 detected in each supernatant from replicate cultures of cells sorted at day 4. A clear pattern of IL-4 secretion related to division number was observed with levels increasing progressively over consecutive divisions 4–7. There was no indication of differential cell death across divisions, as indicated by light scatter profiles of cells analyzed after restimulation (data not shown). Nor was there any evidence of accelerated proliferation by cells in later divisions over the 24 h as indicated by CFSE profiles after restimulation (data not shown).

In addition to IL-4, the cytokines IL-2, IL-3, and IFN-γ were measured. Fig. 2*D* shows that IL-2 secretion was high in the earlier divisions but declined as the cells reached the seventh division cycle. Expression of both IFN-γ (Fig. 2*E*) and IL-3 (Fig. 2*F*) increased in a division-dependent manner with

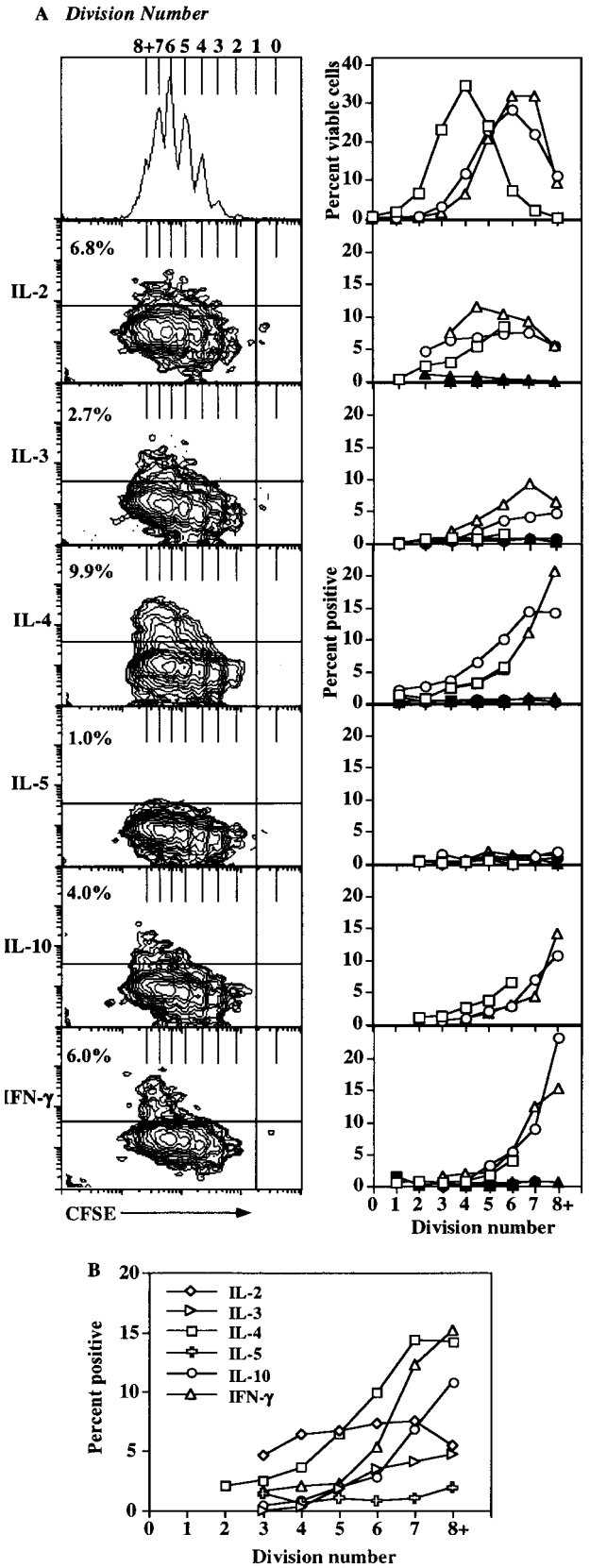

FIG. 3. Intracellular staining reveals changing frequency of secretion with division. Anti-CD3-, IL-2-, and IL-4-stimulated cultures were restimulated after 3, 4, and 5 days then stained intracellularly for cytokines as indicated. (*A*) Contour plots (70% log density) show total positive cells in the top left quadrants. (*Right*) The percentage of positive cells within each division for each cytokine at each time point. The top right panel shows the distribution of total viable cells within each division at each time. Results are shown for day 3 (□), day 4 (○), and day 5 (△). Closed symbols show blocking controls. (*B*) Overlay of graphs showing expression patterns of each cytokine spanning divisions 2–8 at day 4.

IFN-γ being similar to IL-4 whereas IL-3 was increased between divisions 4 and 5 then plateaued from divisions 5–7.

Two other cytokines, IL-5 and IL-10, were consistently detected only from cells that approached the autofluorescent limit of division detection (Fig. 2*G*, day 5 shown). Thus, developmental patterns for the secretion of each of the cytokines assayed (IL-2, IL-3, IL-4, IL-5, IL-10, and IFN-γ) varied according to division cycle number.

The Relationship Between IL-4 Secretion and Division Number Is Not Affected by Time of Culture. If division number serves as a primary regulator of T cell differentiation in the same manner as B cells, then neither the time taken to reach a division, nor the time spent in a division, should affect the cytokine output upon restimulation. To examine this issue, T cells from asynchronously dividing cultures were sorted according to division number. The results from five independent experiments are presented in Fig. 2 *H–K* for cells taken after either 2, 3, 4, or 5 (two preparations shown) days in primary culture. In striking contrast to the variable distribution of cells within divisions at each time (Fig. 2*H*), the level of IL-4 remained a consistent function of division number independent of the day of analysis (Fig. 2*I*).

The pattern of IL-2 secretion was also consistently related to division number rather than time of culture (Fig. 2*J*). After the initial two divisions, high levels of IL-2 were produced during divisions 3 and 4, which declined steadily with further division to low levels by divisions 8+. The level of IFN-γ secretion also was affected by division number, with higher production from the most extensively divided cells (Fig. 2*K*). There was a trend for production of higher levels of IFN-γ at earlier time points, suggesting an additional level of control of this cytokine.

Division-Related Changes in the Frequency of Cytokine-Secreting Cells Demonstrated by Intracellular Staining. The relationship between division-linked changes in cytokine production and the frequency of secreting cells was assessed by staining CFSE-labeled cells intracellularly after restimulation. The time course shown in Fig. 3 reveals division-related variation in the frequency of IL-2-, IL-3-, IL-4-, IL-10-, and IFN-γ-secreting cells. Representative contour plots of cytokine versus CFSE intensity from day 4 of culture are displayed (Fig. 3, *Left)* adjacent to summaries of the observed frequency of cytokine-positive cells at each time (Fig. 3, *Right*). The results confirm the remarkably consistent relationship between cytokine acquisition and division number. Thus, IL-2-secreting cells increased in frequency with each division, reaching a peak at divisions 4 and 5, then declining with further division. The frequency of IL-4-, IL-10-, and IFN-γ-secreting cells each were time independent, with secreting cells becoming detectable at divisions 3–6 and increasing in frequency with each successive division. The number of IL-3 secreting cells also increased after division 3. There appeared to be an increase in observed frequency between days 3 and 5, which may be attributed to a time-related increase in secretion rate, suggested by the fluorescence intensity of intracellular IL-3 staining (data not shown). In contrast to the other cytokines, IL-5-secreting cells were not significantly detectable in these cells, despite strong IL-5 staining of control Th2 cells (data not shown).

DISCUSSION

The validity of the division hypothesis was examined initially by analyzing cells in overlapping divisions taken at different times from an asynchonously dividing population. Division

number was found to profoundly influence the production of all cytokines detected either by stimulating sorted cells from each division (Fig. 2) or by direct intracellular staining (Fig. 3). The striking association between cytokine secretion and division number was clearly independent of time for IL-2, IL-3, IL-4, and IL-10. Time appeared to have an effect on levels of IFN-γ in supernatants but not when measured by the intracellular staining method. The reason for this difference is unknown but may be because of the effect of brefeldin A in the intracellular staining method, preventing the secretion of factors inhibitory to IFN-γ release.

It was possible that cells with the potential to secrete different cytokines divide at varying rates, thereby contributing to variation in cytokine secretion per division. However, investigation of the rate of BrdU incorporation in each division argues strongly against this possibility. Dividing T cells displayed no trend to enhanced BrdU uptake in later divisions that would be consistent with the selection of a rapidly growing cytokine-secreting population. Rather, the major cause of the division asynchrony seen in T cell cultures was variation in the time taken to enter the first division. Thus, T cells that had undergone the most divisions at days 3–5 were descendants of the first cells to enter the first division. Moreover, the widely disparate times of first division onset did not select for a cytokine-secreting population as IL-4 and IFN-γ were not secreted by the fastest cells into division when they had divided only 2–3 times at day 2. Cantrell and Smith (45) have noted that the time spent in G_1 phase of the cell cycle reflects the inherited number of IL-2 receptors on each T cell such that those with the highest levels divide the most rapidly. These high receptor-bearing cells do not overgrow the cultures because random inheritance of receptor numbers by daughter cells ensures a similar fixed average time spent in G_1 for each division round (45, 46). Thus, it appears that the control of division rate is under the influence of stochastic receptor-based events that do not affect T cell differentiation.

Together these data strongly argue for an essential role of cell division in the regulation and modulation of the T cell cytokine repertoire whereby the probability of a change is a constant function of a division number, irrespective of the time it takes to reach that division. As time is unimportant, T cell differentiation induced by IL-4 can be represented as a map showing frequency of cytokine secretion versus division number. An approximation of how this map appears is shown in Fig. 3*B* by superimposing the frequency of cells secreting different cytokines determined by intracellular staining. Despite extensive division overlap in late divisions and the potential for different sensitivity of detection of each cytokine, a clear sequence is apparent. IL-2-secreting cells appeared first at division 1 and peaked in frequency at division 4 before declining. Cells secreting each of the other cytokines required at least three divisions before becoming detectable and appeared in the sequence IL-4, IL-3, IFN-γ, and IL-10. Although not detected by intracellular staining, IL-5 was found in the supernatant taken from the most extensively divided cells, suggesting that acquisition of this cytokine may follow IL-10.

The development of IL-4-secreting cells has been reported to require at least 3–4 days of culture (11, 39, 47). The results reported here show how time acts indirectly, being required to enable cells to progress through sufficient division cycles for the differentiation effects of IL-4 to take place. By the same argument, the observation that cytokine mRNA is expressed in what appears to be a set sequence (40, 48) can be explained by division-dependent regulation, with each cytokine having an independent association with division. It is also possible that at least some of the many variables that have been reported to influence cytokine repertoire *in vitro* (8–18) are acting by altering the passage of cells through different cycles. At least two categories of factors therefore must be distinguished. Primary influences on differentiation would act to alter the relation with division and include IL-4. Secondary influences would alter proliferation and therefore the distribution of cells in each division and may include cytokines that promote T cell activation or IL-2 receptor expression. With the development of CFSE-based methods, the mode of action of each described variable now can be determined and should provide additional insight into the control of T cell behavior.

The overlapping and stochastic nature of division- related cytokine changes is consistent with the detection of multiple patterns of cytokine associations within single cells taken from a population as reported by Kelso *et al.* (5–7). These studies argued strongly that the acquisition of each cytokine was independent even within the same cell (5–7). With the intracellular method used here it will be possible to measure two cytokines simultaneously so that the probabilities of association and how they might change with division can be determined, leading to a more complete description of T cell differentiation.

These data raise questions as to the molecular mechanism by which differentiation is linked to division because they reveal that the cells either are counting in some manner or that some of the differentiation changes responsible for modifying gene expression require passage through S phase of the cell cycle. Future molecular analysis can resolve these issues. However, it is already apparent that the mechanism must have a stochastic component as the link between division number and differentiation is not identical in all cells but is spread over a number of division cycles. Nevertheless, even before the exact mechanism is elucidated, the observations reported here, together with previous data relating to B cells (20, 21) provide insight into how immune response class is regulated. As antigen persistence is required for continued lymphocyte proliferation, a feedback is suggested for both T and B cells whereby different functional strategies are linked to the successful clearance of a pathogen. If the early class of response is unsuccessful and antigen persists, a new strategy is applied. In each case, continued division and exposure to antigen will be the trigger for such change.

It may seem that the production of individual cytokines occurs too close together in relation to division to allow any control based on division sequence. However, previous analysis of division-related changes in isotype switching by B cells argues against this conclusion. Thus, lowering the dose of IL-4 extended the number of divisions required before isotype switching occurred, serving to spread the changes over a broader number of divisions (20, 21). As it is unlikely that T cell proliferation *in vivo* occurs under saturating concentrations of differentiation factors, the number of divisions between changes in cytokine profile is likely to be much greater than that suggested by Fig. 3*B*. Importantly, however, the sequence of cytokines will be retained.

Examination of our data suggests that an evolution-imposed logic may exist in the sequence in which cytokines are produced. For example, IL-10 production was seen only after an extensive number of division rounds. Hence, acquisition of this cytokine may act as a cue warning the host that despite extensive clonal expansion the antigen continues to persist and a separate course of action should be taken. Other T cells will not have advanced to the same number of divisions and will continue to stimulate an ineffective, but potentially dangerous, inflammatory response. Therefore, IL-10 as a potent inhibitor of IFN-γ (49) could feed back from the most divided cells to prevent the less divided cells from causing further damage. Consistent with such a conclusion is the observation that chronic bowel inflammation occurs to persisting common intestinal antigens in hosts lacking IL-10 (50). A second example is IL-5, which also was found only in late divisions. This cytokine plays an important role in activating and increasing the number of eosinophils needed for protection against helminths (51). Because these parasites present no

acute difficulty to the host it is logical to mount this type of response late. Therefore, faced with a new invasion of unknown potential, the eosinophil-mediated antiparasite response is pursued only after the earlier inflammatory attack has failed. We anticipate that further mapping of division-based differentiation under other stimulatory conditions will reveal additional logic encoded within the T cell cytokine response.

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