whether ubiquitination of transcription factors triggers recruitment of APIS to genes that are to be transcribed.

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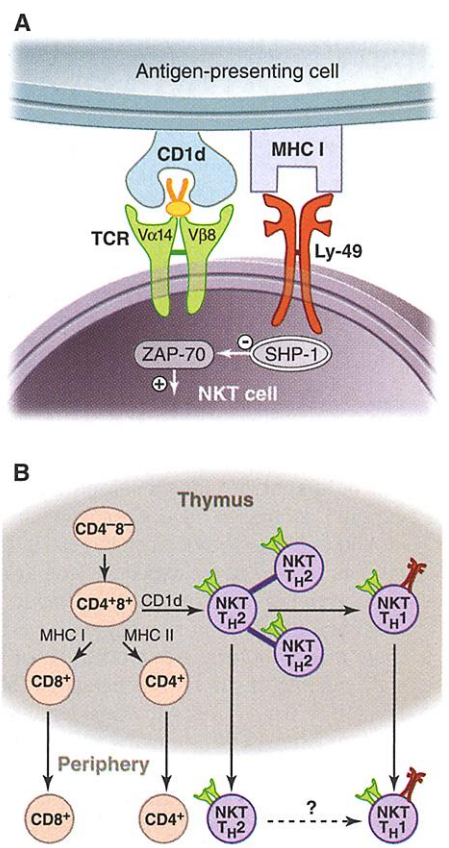
T Before NK

H. Robson MacDonald

Natural killer T (NKT) cells are a minor subset of mature lymphocytes that, as their name suggests, express receptors associated with both T cell and NK cell lineages. They have attracted a lot of attention because they regulate not only autoimmunity but also immune responses against microbes and tumors (*1*, *2*). NKT cells are able to carry out this wide array of tasks partly because, when activated, they secrete large amounts of cytokines, such as interferon-γ (IFN-γ) and interleukin-4 (IL-4). These hybrid lymphocytes express a heterodimeric αβ T cell receptor (TCR), as well as the NK cell receptors (NKRs) NK1.1 and members of the Ly-49 receptor family. When expressed by T cells, TCR is an activating receptor that transduces positive signals through mobilization of intracellular tyrosine kinases. In contrast, Ly-49 receptors expressed by NK cells are inhibitory receptors that recruit intracellular phosphatases, which dephosphorylate and hence inactivate kinases (see the figure, panel A). Thus, the coexpression of TCR and Ly-49 receptors by NKT cells raises the intriguing question of how these potentially opposing receptors are regulated during NKT cell development. Enter Benlagha *et al.* (*3*) on page 553 of this issue and Pellicci *et al.* (*4*) in the *Journal of Experimental Medicine* with a solution to this dilemma. Both groups identify a new intermediate cell in the NKT lineage that reveals how TCRs and NKRs operate during differentiation and maturation of NKT cells.

It is now generally accepted that most (if not all) NKT cells, like conventional T cells, originate in the thymus. However, the search for immature NKT lineage precursors in the thymus is complicated by the fact that mature NKT cells represent a very small fraction (0.3 to 0.5%) of total thymic T cells (thymocytes). To overcome this problem, Benlagha *et al.* (*3*) and Pellicci *et al.* (*4*) have exploited a recent technical advance that allows the direct identification of NKT lineage cells by virtue of their restricted TCR specificity. Whereas conventional T cells express diverse TCRs that recognize short peptides in association with highly polymorphic major histocompatibility complex (MHC) molecules, the highly conserved TCR on NKT cells exclusively recognizes glycolipids bound to the monomorphic CD1d molecule (see the figure, panel A). Although the endogenous and foreign glycolipids recognized by the TCR on NKT cells under physiological conditions are not known, a synthetic glycolipid (α-galactosyl ceramide) bound to CD1d mimics their effects. Hence, by preparing fluorescent tetramers of α-galactosyl ceramide bound to CD1d it is possible to track very small numbers of NKT lineage cells by flow cytometry (*5*, *6*).

With this approach, Benlagha *et al.* (*3*) and Pellicci *et al.* (*4*) identified a new subset of thymocytes that bind to CD1d tetramers but do not express NKRs, such as NK1.1 and members of the Ly-49 inhibitory receptor family. When purified and injected directly into the thymus of a genetically marked syngeneic recipient mouse, NKR^- $tetramer^+$ cells gave rise to NKR^+ $tetramer^+$ cells in both the thymus and peripheral lymphoid tissues. This observation provided direct evidence for a lineage relationship between these two populations. Surprisingly, quantitation of NKR^- and NKR^+ subsets of $tetramer^+$ cells among recent thymic emigrants (using in situ fluorescein isothio-



Balancing act. (**A**) NKT cells express a conserved αβ TCR (composed of Vα14 and Vβ8 chains) that recognizes glycolipids bound to CD1d, as well as inhibitory Ly-49 receptors that bind MHC class I molecules. Whereas TCR ligation leads to activating signals mediated by tyrosine kinases (such as ZAP-70), engagement of Ly-49 receptors recruits phosphatases (such as SHP-1) that can potentially neutralize kinase activity. (**B**) During T cell development in the thymus, $CD4^-8^-$ precursor cells give rise to immature $CD4^+8^+$ thymocytes that randomly express TCRs. $CD4^+8^+$ thymocytes expressing TCRs with appropriate affinity for MHC class I and II molecules, respectively, develop further into conventional $CD8^+$ and $CD4^+$ T cells. Rare $CD4^+8^+$ thymocytes expressing TCRs that bind to CD1d develop along the NKT cell lineage, first undergoing proliferation and subsequently expressing inhibitory NKRs such as Ly-49. During this maturation process the cytokine-producing potential of NKT cells evolves from a T_H2 to a T_H1 pattern. Both immature and mature NKT cells can be exported from the thymus to the periphery, but it is not known whether NKT cell maturation can take place outside of the thymus.

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cyanate labeling) revealed that the former population is preferentially exported to the periphery. Taken together with other recent data (*7*) indicating that mature NKT cells are ultimately derived from a $CD4^+$ $CD8^+$ thymic subset (which also contains precursors of conventional $CD4^+$ and $CD8^+$ T cells), a plausible model for the intrathymic development and export of NKT cells can now be proposed (see the figure, panel B).

What are the possible implications of this model for the respective parts played by NKRs and TCRs during NKT cell development? In this context, Benlagha *et al.* (*3*) present a key finding: The NKR^- NKT cell intermediate divides rapidly in the thymus, whereas the more mature NKR^+ progeny do not. An interesting interpretation of these data would be that TCR interactions with CD1d on immature NKT lineage cells lead to activation and proliferation of these cells; in contrast, the delayed expression of inhibitory NKRs dampens this proliferative response and prevents potential autoreactivity of mature NKT cells. Intriguingly, engagement of Ly-49 receptors with ligand negatively regulates autoreactivity of NKT cells mediated by TCRs (*8*). Furthermore, enforced ligation of Ly-49 receptors in transgenic mice can interfere with NKT cell development (*9*). Thus, the "T before NK" strategy of receptor expression adopted by developing NKT cells appears to be a way of optimally exploiting the unique properties of each cell lineage.

Benlagha *et al.* (*3*) and Pellicci *et al.* (*4*) also examined the cytokine profile of developing NKT cells. The accepted paradigm (*10*) for conventional $CD4^+$ T cells is that they can be induced to differentiate into either T helper 1 (T_H1) cells, which produce preferentially IFN-γ and related "inflammatory" cytokines, or T_H2 cells, which produce preferentially IL-4 and related "regulatory" cytokines. Mature NKT cells produce large amounts of both IFN-γ and IL-4 and thus do not readily fit the T_H1/T_H2 classification. Surprisingly, it turns out that immature NKR^- NKT cells produce much more IL-4 than IFN-γ, whereas in mature NKR^+ NKT cells this balance reverts in favor of IFN-γ. Because both NKR^- and NKR^+ NKT cells are exported from the thymus to the periphery, it is possible that NKT cells may mediate either T_H1 or T_H2 responses, depending upon which NKT cell subset is preferentially activated.

The work of Benlagha *et al.* (*3*) and Pellicci *et al.* (*4*) has far-reaching implications for understanding NKT cell biology. These studies may define a more general paradigm for the development and selection of lymphocytes of the innate immune system that reaches beyond NKT cells to include NK cells, epidermal γδ T cells, and the B-1 subset of B cells (*11*). In contrast to conventional T and B cells of the adaptive immune system, these innate lymphocytes all express germline–encoded activating receptors of limited (or no) diversity, as well as inhibitory NKRs. Because innate lymphocytes are expanded (rather than eliminated) by strong agonist interactions with conserved self ligands, it is tempting to speculate that delayed expression of inhibitory receptors during development may represent an evolutionarily conserved mechanism to terminate expansion and to control autoreactivity of these cells.

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PERSPECTIVES: PARASITOLOGY

When the Host Is Smarter Than the Parasite

Daniel E. Goldberg

The malaria parasite, *Plasmodium falciparum*, causes millions of deaths each year, mostly among African children (*1*). Drug resistance is rendering the standard affordable agents like chloroquine obsolete. We desperately need new drugs, and toward that end, new drug targets. To the rescue has come the malaria genome-sequencing consortium. The *P. falciparum* genome sequence is nearing completion, and we are sure to turn up new drug targets among the 6000 or so genes of the malaria parasite.

The first place to look is the set of genes (perhaps 60% of the *P. falciparum* genome) that exist in the parasite but not in its human host (*2*, *3*). Most of these will prove to be nonessential—for example, only 14% of yeast genes without mammalian homologs are essential (*4*). Most of the remainder are of unknown function, and it will be a challenge to develop drugs directed against their gene products. Still others may be difficult to express or screen and may not have a potent nontoxic bioavailable inhibitor that can be taken through development. The genome databases have already been exploited to identify good drug targets and inhibitors (*5*, *6*), but the expected drug development riches may amount to a trickle instead of a flood. Soon, we may wish we had more than 6000 genes to work with in the fight against this nefarious parasite. There is another list of genes to consider: Those that are expressed in humans as well as in malaria organisms. Proteins encoded by some of these genes exhibit sufficient differences between man and malaria parasite that they could be exploited as targets for drug development. Many are very similar between the two species, especially in their active sites. Are we to discard these as potential drug targets? According to the study by Zhang and Rathod on page 545 of this issue (*7*), the answer is "not so fast."

Dihydrofolate reductase (DHFR), a key enzyme in the folate metabolic pathway, is an important target for antimicrobial chemotherapeutics such as pyrimethamine, one of the most enduring of antimalarial drugs. Selectivity for pathogen DHFR over the host enzyme is usually the key feature of DHFR inhibitors. Yet, for some agents such as WR92210, the inhibition constants (K_i's) for the human and parasite enzyme differ by a mere order of magnitude; in contrast, cellular toxicity differs by five orders of magnitude. Zhang and Rathod have now explained this discrepancy with a simple but elegant model in which the host, but not the parasite, is able to overcome drug toxicity by making more enzyme (*7*).

There exists in both host and parasite a negative-feedback loop in which mRNA encoding DHFR is bound by its protein, preventing further translation of the mRNA once enough protein has been made. In mammalian cells, when folate substrate levels rise, DHFR mRNA becomes dissociated

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