A New Look at Syk in ab and gd T Cell Development Using Chimeric Mice with a Low Competitive Hematopoietic Environment

Francesco Colucci, Delphine Guy-Grand, Anne Wilson, Martin Turner, Edina Schweighoffer, Victor L. J. Tybulewicz and James P. Di Santo

J Immunol 2000; 164:5140-5145; ;
doi: 10.4049/jimmunol.164.10.5140
http://www.jimmunol.org/content/164/10/5140

A New Look at Syk in $\alpha\beta$ and $\gamma\delta$

development of precursors during fetal life (6). Allophenic chimeras generated by aggregating *Syk*$^{-/-}$ and recombination-activating gene (RAG) 2$^{-/-}$ morulae confirmed the essential role for Syk in DETC development, but also found a severe reduction in gut IEL γδ T lymphocytes (in contrast to splenic γδ T cells), suggesting that these γδ T cells also rely on Syk (12). *Zap70*$^{-/-}$ mice also fail to generate intestinal γδ T lymphocytes and develop morphological abnormal DETC, while other γδ T lymphocytes develop relatively normally (13). Altogether, these results indicate that γδ T cell subsets may have differential requirements for Syk family PTKs.

The RAG2$^{-/-}$ blastocyst complementation system has facilitated the study of genes involved in lymphoid development (14). However, results obtained using somatic chimeras generated by irradiation and hematopoietic stem cell (HSC) reconstitution have to be interpreted with caution, especially with regard to the lack of development of a given lymphocyte subset. Simply stated, when the donor (i.e., mutant) cell population is confronted by the host one, the two lymphoid populations will compete for growth factors and developmental niches. Among HSCs, it may be that a committed progenitor has a selective disadvantage due to the lack of a given gene product and therefore may be competed out by the host progenitors and fail to differentiate further. This may be the case for intraepithelial γδ T cells deriving from *Syk*$^{-/-}$ HSCs which do not develop in *Syk*$^{-/-}$ allophenic chimeras in the RAG2$^{-/-}$ background (12). In a situation where the host progenitors are fewer or are impaired in their own differentiation program, intraepithelial γδ T cells may be generated from *Syk*$^{-/-}$ HSCs. We have developed a novel alymphoid mouse strain by combining RAG2 and common cytokine receptor γ-chain (γc) mutations (RAG2/γc$^{-/-}$ mice (15)). The absence of lymphoid progenitors in RAG2/γc$^{-/-}$ mice provides a situation where competition from host cells should be negligible. Using this system, we have re-evaluated the effects of the Syk deficiency on T cell development.

Materials and Methods

Mice and generation of hematopoietic chimeras

C57BL/6, RAG2$^{-/-}$ (RAG2; Ref. 16) and RAG2$^{-/-}$/γc$^{-/-}$ (RAG2/γc; Ref. 15) mice were maintained in specific pathogen-free conditions at a cribcilit2 1.390 261.t2 1257 Tt2 1morg

RAG2 chimeras (Table I and Fig. 1*A*). A plausible explanation for the lower thymic reconstitution of RAG2 mice by *Syk*$^{-/-}$ FL-HSC relates to the higher numbers of early lymphoid precursors present in RAG2 mice (15). Indeed, most cells found in the thymi of *Syk*$^{-/-}$ → RAG2 chimeras were CD4^{-}CD8^{-} (double negative (DN); see Fig. 1*A*) that were host derived (negative for H-2^{d}; Fig. 1*B*). Furthermore, wt FL-HSC generated 100-fold greater total thymocyte numbers in the RAG2 recipient mice compared with the *Syk*$^{-/-}$ FL-HSC (Table I), despite the presence of host RAG2 DN cells, whereas only 2-fold fewer thymocytes were detected in RAG2/γc recipient mice generated from *Syk*$^{-/-}$ FL-HSC (Table I and Fig. 1*A*). These results demonstrate that Syk-deficient HSC can only poorly compete against the resident RAG2 thymic precursors and suggest a novel role for Syk in early T lymphoid development, which could be appreciated in the competitive RAG2 environment, using this irradiation protocol (0.3 Gy).

We further analyzed early T cell development in the absence of Syk (Fig. 2). For this purpose, early T cell precursors (defined as CD3^{-}CD4^{-}CD8^{-}TCR$\alpha\beta^{-}$TCR$\gamma\delta^{-}$B220^{-} thymocytes) from wt or *Syk*$^{-/-}$ FL-HSC → RAG2/γc chimeras were stained for expression of CD44 and CD25. Previous studies have shown that immature thymocytes differentiate along the following pathway: CD44^{+}CD25^{-} → CD44^{+}CD25^{+} → CD44^{-}CD25^{+} → CD44^{-}CD25^{-}

(reviewed in Ref. 30). Previous studies have reported that the intestinal intraepithelial γδ T cells are severely reduced in *Syk*$^{-/-}$ aggregation chimeras (12). We hypothesized that competition with host lymphoid precursors might have blocked Syk-deficient IEL development in those experiments and, to test this hypothesis, we compared reconstitution of the intestinal IEL pool in RAG2 and RAG2/γc mice injected with FL-HSC (Fig. 4). Although it is difficult to accurately quantitate numbers of IELs, they are best expressed as a ratio to epithelial cells. Overall IEL development was similar in wt and *Syk*$^{-/-}$ FL-HSC → RAG2/γc chimeras, with an average of 14–15 IELs/100 epithelial cells. Almost half of the IELs were defined as γδ T cells in both RAG2 and RAG/

13. Kadlecek, T. A., N. S. C. van Oers, L. Lefrancois, S. Olson, D. Finlay, D. H. Chu, K. Connolly, N. Kileen, and A. Weiss. 1998. Differential requirements for ZAP-70 in TCR signaling and T cell development. *J. Immunol. 161:4688.*
14. Chen, J., R. Lansford, V. Stewart, F. Young, and F. W. Alt. 1993. RAG-2-deficient blastocyst complementation: an assay of gene function in lymphocyte development. *Proc. Natl. Acad. Sci. USA 90:4528.*