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A New Look at Syk in $\alpha\beta$ and $\gamma\delta$ T Cell Development Using Chimeric Mice with a Low Competitive Hematopoietic Environment[1]

Francesco Colucci,[2]*† Delphine Guy-Grand,* Anne Wilson,‡ Martin Turner,§ Edina Schweighoffer,¶ Victor L. J. Tybulewicz,¶ and James P. Di Santo*†

The Syk protein tyrosine kinase (PTK) is essential for B, but not T or NK, cell development, although certain T cell subsets (i.e., $\gamma\delta$ T cells of intestine and skin) appear to be dependent on Syk. In this report, we have re-evaluated the role of Syk in T cell development in hematopoietic chimeras generated by using Syk-deficient fetal liver hematopoietic stem cells (FL-HSC). We found that *Syk*$^{-/-}$ FL-HSC were vastly inferior to wild-type FL-HSC in reconstituting T cell development in recombinant-activating gene 2 (RAG2)-deficient mice, identifying an unexpected and nonredundant role for Syk in this process. This novel function of Syk in T cell development was mapped to the CD44^{-}CD25^{+} stage. According to previous reports, development of intestinal $\gamma\delta$ T cells was arrested in *Syk*$^{-/-}$ $\rightarrow$RAG2$^{-/-}$ chimeras. In striking contrast, when hosts were the newly established alymphoid RAG2 × common cytokine receptor γ-chain (RAG2/γ_c) mice, *Syk*$^{-/-}$ chimeras developed intestinal $\gamma\delta$ T cells as well as other T cell subsets (including $\alpha\beta$ T cells, NK1.1^{+} $\alpha\beta$ T cells, and splenic and thymic $\gamma\delta$ T cells). However, all Syk-deficient T cell subsets were reduced in number, reaching about 25–50% of controls. These results attest to the utility of chimeric mice generated in a low competitive hematopoietic environment to evaluate more accurately the impact of lethal mutations on lymphoid development. Furthermore, they suggest that Syk intervenes in early T cell development independently of ZAP-70, and demonstrate that Syk is not essential for the intestinal $\gamma\delta$ T cell lineage to develop. ***The Journal of Immunology*, 2000, 164: 5140–5145.**

The Syk and ZAP-70 protein tyrosine kinases (PTK)[3] form a family of signal-transducing molecules required for normal hematopoietic development. By virtue of their tandem Src homology 2 domains, Syk and ZAP-70 PTKs associate with tyrosine-phosphorylated immunoreceptor tyrosine-based activation motifs contained within the cytoplasmic domains of activating cell surface receptors, including the B and T cell Ag receptors and the Fc receptors for IgG and IgE (reviewed in Refs. 1 and 2). Initial activation of ZAP-70 (but not Syk) requires a src family kinase such as lck or fyn (3, 4). Subsequent Syk- or ZAP-70-dependent phosphorylation of cellular substrates (including LAT and SLP-76 in T and NK cells and BLNK in B cells) is necessary for signal transduction through the relevant receptors. The modulation of Syk/ZAP-70 activity (perhaps through negative regulators like Cbl) may result in the formation of different intracellular adaptor protein complexes and thereby offer a mechanism to regulate biological responses.

Previous reconstitution experiments using Syk- or ZAP-70-deficient cells have identified the essential roles for these molecules during normal development in vivo. Although mice deficient in ZAP-70 (*Zap70*$^{-/-}$) are viable (5), *Syk*$^{-/-}$ mice die in the perinatal period from excessive hemorrhage (6, 7). B cells strictly rely on Syk to transduce signals through the Ig receptor and in the absence of Syk, B cell development is partially blocked at the pro-B cell stage and completely blocked at the pre-B cell stage (6, 8, 9). In contrast, $\alpha\beta$ T cell development appears to be Syk-independent (6, 7). Reciprocally, T cells require ZAP-70 association with the CD3 complex to transduce TCR (but not pre-TCR) signals. In mice, ZAP-70-deficient thymocytes develop only to the CD4^{+}CD8^{+} double-positive (DP) stage, whereas B cell development in *Zap70*$^{-/-}$ mice is completely normal (5). Functional NK cells develop in the absence of either Syk (9) or ZAP-70 (5). Thus, the Syk/ZAP-70 PTKs appear to have unique roles in B cells and $\alpha\beta$ T cells and redundant roles in NK cells, which likely reflect the differential patterns of Syk and ZAP-70 expression in these lymphoid subsets (10).

Subsets within the $\gamma\delta$ T cell lineage have been characterized based on their appearance in ontogeny, their usage of certain TCR variable gene segments, and their ultimate anatomical localization (reviewed in Ref. 11). The $\gamma\delta$ T cells that home preferentially to epithelial tissues include the skin dendritic epidermal T cells (DETC) and the intraepithelial lymphocytes (IELs) associated with the digestive tract (11). A number of reports have investigated the effects of Syk or ZAP-70 deficiency on the development of these $\gamma\delta$ T cell subsets, the reduction being likely to relate to abnormal

*Institut National de la Santé et de la Recherche Médicale U429, Hôpital Necker-Enfants Malades, Paris, France; †Laboratory for Cytokines and Lymphoid Development, Institut Pasteur, Paris, France; ‡Ludwig Institute for Cancer Research, Eplainger, Switzerland; §Babraham Institute, Babraham, Cambridge, United Kingdom; and ¶National Institute for Medical Research, London, United Kingdom

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[2] Address correspondence and reprint requests to Dr. Francesco Colucci, Laboratory for Cytokines and Lymphoid Development, Institut Pasteur, Paris, France, 25 rue du Docteur. Roux, 75015, Paris, France. E-mail address: cecco@pasteur.fr

[3] Abbreviations used in this paper: PTK, protein tyrosine kinase; IEL, intraepithelial lymphocyte; RAG, recombination-activating gene; γc, common cytokine receptor γ-chain; HSC, hematopoietic stem cell; FL-HSC, fetal liver HSC; DETC, dendritic epidermal T cell; wt, wild type; LN, lymph node; SP, single positive; DP, double positive; DN, double negative; TRIC, Tricolor.

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Table I. *Thymic cellularity of RAG2/γ_c and RAG2 mice reconstituted with FL cells*[a]

Host	Donor	Total (10^6)	DP[b] (10^6)	CD4 SP (10^6)	CD8 SP (10^6)	DN (10^6)	$\gamma\delta$T[c] (10^4)	NK T[d] (10^4)	*n*
RAG2/$\gamma_c^{-/-}$	wt	90 ± 28	72 ± 22	3.9 ± 1.4	1.2 ± 0.5	0.5 ± 0.2	15 ± 10	18 ± 6	5
RAG2/$\gamma_c^{-/-}$	*Syk*$^{-/-}$	45 ± 26	34 ± 19	2.7 ± 1.5	0.8 ± 0.4	0.3 ± 0.1	4 ± 2	9.6 ± 2	7
RAG2$^{-/-}$	wt	103 ± 56	75 ± 34	7.9 ± 4	1.9 ± 0.4	1.9 ± 0.4	10 ± 8	26 ± 17	3
RAG2$^{-/-}$	*Syk*$^{-/-}$	1.3 ± 0.5	0.3 ± 0.1	0.2 ± 0.2	0.3 ± 0.3	0.3 ± 0.2	0.07 ± 0.01	0.6 ± 1	3

[a] Thymi were explanted 8–12 wk after transfer, and cell suspension was enumerated. After calculating the percentage of lymphoid cells (about 85% of total) on the basis of their forward light scatter/side scatter profiles, the numbers of the indicated population were calculated. Both host-derived and donor-derived cells were included in the calculations.
[b] DP: CD8$^+$CD4$^+$ double-positive thymocytes.
[c] $\gamma\delta$T cells were calculated after gating off all cells positive for CD8, CD4, and B220 (>98% of total thymocytes).
[d] NK T cells were calculated after gating off heat-stable antigen-positive immature thymocytes (about 95% of total).

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 $\gamma\delta$ T lymphocytes (in contrast to splenic $\gamma\delta$ T cells), suggesting that these $\gamma\delta$ T cells also rely on Syk (12). *Zap70*$^{-/-}$ mice also fail to generate intestinal $\gamma\delta$ T lymphocytes and develop morphological abnormal DETC, while other $\gamma\delta$ T lymphocytes develop relatively normally (13). Altogether, these results indicate that $\gamma\delta$ 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 $\gamma\delta$ 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 $\gamma\delta$ 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 barrier facility (Centre de Développement des Techniques Avancées/Centre National de la Recherche Scientifique, France) and mice older than 6 wk of age were used as recipients for lymphoid reconstitution using day 16 fetal liver cells from Syk-deficient and wild-type control (*Syk*$^{+/+}$ or *Syk*$^{+/-}$) embryos as described (10). The morning of the vaginal plug discovery was designated as day 0. RAG2 and RAG2/γc mice were irradiated with 0.3 Gy from a cobalt source and 4 h later were injected i.v. with fetal liver cells as described elsewhere (10). All mice received tetracycline and bactrim in the drinking water for the period following the fetal liver cell transfer.

Flow cytometry

Single-cell suspensions were prepared from spleen, thymus, liver, and intestine epithelium as described previously (15, 17). Erythrocytes were lysed in ammonium chloride and cells were resuspended in PBS with 3% FCS and 0.01% sodium azide. mAbs directly conjugated to FITC, PE, Tricolor (TRIC), or biotin were used for immunofluorescence analysis, as described previously (15), including mAbs specific for CD3, CD4, CD8, CD25, CD44, B220, IgM, H-2K^d, TCR$\alpha\beta$, TCR$\gamma\delta$, HSA, and NK1.1 (all from PharMingen, San Diego, CA).

Cell isolation and in situ hybridization

In situ hybridization was done as described elsewhere (18). Briefly, thymi and lymph nodes were explanted from normal C57BL/6 mice. CD4$^-$CD8$^-$CD3$^-$ thymocytes were prepared by complement-mediated depletion with anti-CD4 and anti-CD8 Abs followed by Dynabead (Dynal, Compiègne, France) depletion with anti-CD3 Abs to remove all mature cells. CD44$^+$ CD25$^-$, CD44$^+$CD25$^-$, CD44$^+$CD25$^-$, and CD44$^+$CD25$^-$ thymic subsets, CD3$^+$ lymph node (LN) T cells or B220$^+$ LN B cells, CD4$^+$CD8$^+$ (DP), CD4$^-$CD8$^+$CD3$^-$ immature single positive (SP), and CD4$^-$CD8$^+$CD3$^+$ (SP) cells were sorted directly onto poly L-lysine-coated slides by FACS. Where possible, positive and/or negative cell subsets were sorted onto the same slides as the test subsets. After fixation, proteinase K treatment, and acetylation, specific mRNA transcripts were detected by hybridization with ^{35}S-UTP labeled RNA probes as follows: for ZAP-70, a 700-bp *Eco*RI/*Acc*I fragment of the mouse cDNA was cloned into pSP73; for Syk, a 700-bp *Xba*I/*Eco*RI fragment was cloned into pBluescript SK$^+$. Sense and antisense probes were transcribed with T7, T3, or SP6 RNA polymerases after linearization with the appropriate restriction enzymes. Results are the means ± SD of at least three independent experiments, two separate slides for each subset per experiment and 200–500 cells per slide were counted. In all cases, both antisense and sense probes were used for each subset and the background with the sense probe was subtracted.

Results and Discussion

A novel role for Syk in early T cell progenitors

We compared lymphoid reconstitution in sublethally irradiated (0.3 Gy) RAG2 vs RAG2/γc mice (H-2^b) reconstituted with day 16 fetal liver HSC (FL-HSC) from wt or Syk-deficient embryos (H-2^d). RAG2/γc may represent a better system to analyze lymphoid development arising from mutant stem cell precursors (19), since they are severely depleted in lymphoid precursors (15), therefore making a lower competitive environment to the donor FL-HSC. Sublethal irradiation was chosen to avoid the development of hemorrhagic ascites that has been observed in lethally irradiated RAG2 mice reconstituted with Syk-deficient HSC (20). Eight to 12 wk after transfer, we analyzed bone marrow, thymic, splenic, hepatic, and IEL and quantitated the numbers of B and T cells (including $\alpha\beta$, $\gamma\delta$, and NK1.1$^+$ T cells).

The results for thymocytes are summarized in Table I and Fig. 1. Concerning hematopoietic reconstitution using wt FL-HSC, no differences in the overall lymphoid cellularity or distribution of lymphocytes subsets were detected between RAG2 and RAG2/γc chimeras (Fig. 1*A*), suggesting that the higher competitive environment in RAG2 mice does not impede wt HSC cells to fully reconstitute immunodeficient mice. In contrast, a large impact of the host environment was seen in chimeras reconstituted with Syk-deficient HSC: an average of roughly 40-fold more thymocytes was found in *Syk*$^{-/-}$ → RAG2/γc chimeras than in *Syk*$^{-/-}$ →

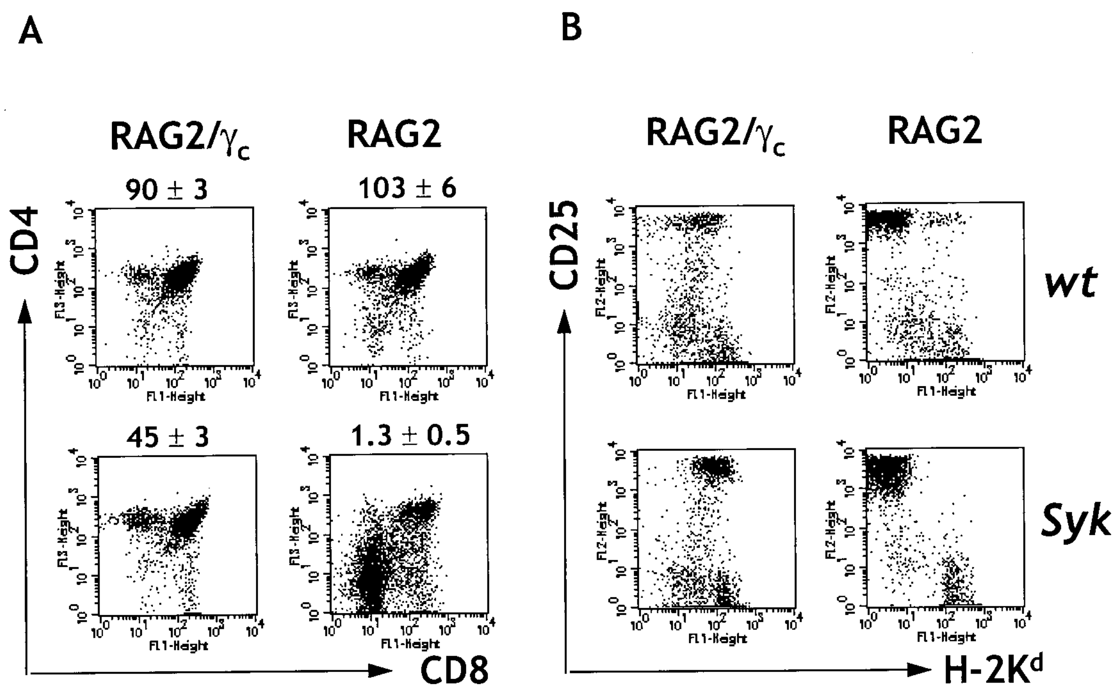

FIGURE 1. Thymic reconstitution by wt and *Syk*$^{-/-}$ FL-HSC in RAG2 vs RAG/γc chimeras. The thymi were explanted from the indicated mice 8–12 wk after transfer. Absolute numbers of total thymocytes (and SD) are expressed in millions. Cell suspensions were stained with mAbs specific for either CD8-FITC and CD4-TRIC (A) or for CD4-TRIC, CD8-TRIC, CD25-PE, and H-2^d-FITC (*B*). For the CD25/H-2^d staining, a live electronic gate was set to exclude CD4$^+$CD8$^+$ cells, and data are presented for the CD4$^-$CD8$^-$ (DN) thymocytes. wt and *Syk*$^{-/-}$ refer to the genotype of the donor FL cells. Data are from one representative of three independent experiments.

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*$^{-/-}$ $\rightarrow$ 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 $\rightarrow$ 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$^-$ $\rightarrow$ CD44$^+$CD25$^+$ $\rightarrow$ CD44$^-$CD25$^+$ $\rightarrow$ CD44$^-$CD25$^-$ (21). Compared with wt chimeras, Syk-deficient thymocyte precursors demonstrated an accumulation of CD44$^-$CD25$^+$ cells (Fig. 2). Although we did not exclude the host-derived cells from the analysis, a possible contribution to the detected difference between wt and *Syk*$^{-/-}$ early thymocytes is unlikely, since RAG/γc thymi contain only a few thousand lymphoid cells in total (15). Pre-T cells at this stage are actively rearranging TCR β, γ, and δ gene segments (22, 23) and productive assembly of either a $\gamma\delta$ TCR or pre-TCR (composed of the invariant pTα-chain and a rearranged TCRβ-chain) presumably signals the cell for further differentiation via ZAP-70 and Syk (24). The partial block observed in CD44$^-$CD25$^+$ cells from *Syk*$^{-/-}$ FL-HSC $\rightarrow$ RAG2/γc chimeras could be explained if ZAP-70 were absent in these cells, since thymocytes deficient in both Syk and ZAP-70 arrest at the this stage (24). To test this, we performed in situ hybridization experiments to characterize ZAP-70 or Syk expression in early thymocyte subsets. As shown in Table II, ZAP-70 and Syk are coexpressed throughout early thymopoiesis, including the pre-T cell stage, whereas ZAP-70 becomes the dominant Syk family PTK from the DP stage onward, concomitant with the down-regulation of Syk. In line with this, Chu et al. (25) have shown that Syk protein is down-regulated after the pre-TCR

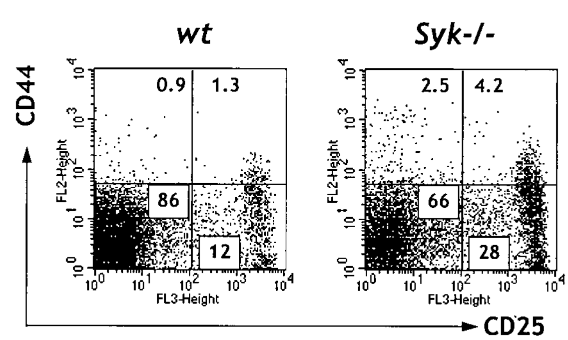

FIGURE 2. Early T cell development in RAG/γc chimeras. The thymi were explanted from the indicated mice 8–12 wk after transfer. Cell suspensions were stained with mAbs specific for CD3, CD4, CD8, TCR$\alpha\beta$, TCR$\gamma\delta$, and B220, all conjugated to FITC, CD44-PE, and CD25 biotinylated, followed by streptavidin-TRIC. A live electronic gate was set to exclude FITC$^+$ thymocytes, and the percentages of the indicated populations are indicated. wt and *Syk*$^{-/-}$ refer to the genotype of the donor FL cells. Data are from one representative of three independent experiments.

Table II. *Expression of Syk and ZAP-70 in early thymocytes by in situ hybridization*[a]

Subset	Syk	ZAP-70
$CD44^+CD25^-$	74 ± 10	18 ± 4
$CD44^+CD25^+$	30 ± 4	18 ± 5
$CD44^-CD25^+$	22 ± 8	29 ± 5
$CD44^-CD25^-$	40 ± 5	57 ± 10
ISP[b]	6 ± 3	37 ± 10
DP	4 ± 3	28 ± 10
SP $CD8^+$	4 ± 3	25[c]
LN T	7 ± 4	29 ± 8
LN B	79 ± 2	5[c]
γδ T	4 ± 2	68[c]

[a] Thymi and LN were explanted from adult B6 mice. The indicated subsets were isolated by FACS sorting and cells were analyzed for Syk and ZAP-70 expression with specific probes. Mean percentages ± SD of positive cells for three independent experiments (unless otherwise stated) are indicated.
[b] ISP, immature single positive.
[c] One experiment only.

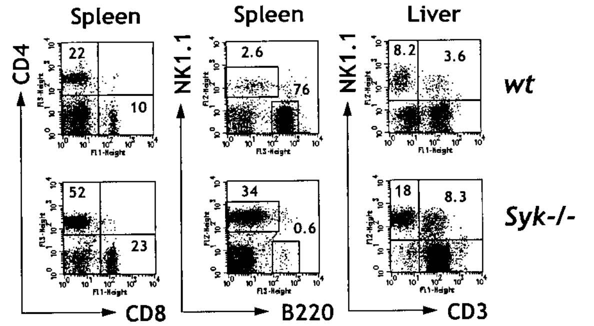

FIGURE 3. Peripheral lymphoid development in RAG/γc chimeras. The spleens and livers were explanted from the indicated mice 8–12 wk after transfer. Cell suspensions were stained with mAbs specific for either CD4 and CD8 or CD3, NK1.1, and B220. Host-derived cells (H-2^{b+}) expressing these markers are absent (data not shown), and acquired events are gated on lymphoid cells, which are all donor derived. wt and *Syk*$^{-/-}$ refer to the genotype of the donor FL cells. Percentages of boxed populations are indicated. Data are from one representative of five independent experiments.

checkpoint has been passed. These results suggests that pre-T cells have the capacity to signal via both ZAP-70 and Syk. However, Syk-deficient pre-T cells are impaired in their development, despite expressing ZAP-70 (Fig. 2 and Table II). Considering that Syk and ZAP-70 have different requirements for activation (26, 27), Syk may subserve functions distinct from ZAP-70 in early T cell development in vivo.

The observation that *Syk*$^{-/-}$ FL-HSC are inferior to wt FL-HSC in competing against residual host thymocyte elements in RAG2 mice is consistent with a nonredundant role for Syk in early T cell development. The transitional block of Syk-deficient $CD44^-CD25^+$ cells in RAG2/γc chimeras would corroborate the idea that pre-TCR or γδ TCR signaling is preferentially propagated through Syk and not ZAP-70. It remains possible that subpopulations of $CD44^-CD25^+$ cells differentially express ZAP-70 and Syk proteins, whereas the partial block in Syk-deficient early thymocyte differentiation might reflect loss of those cells which fail to coexpress ZAP-70. Alternatively, ZAP-70 expression may have to exceed a certain threshold level to fully compensate for the absence of Syk. The development of techniques to simultaneously evaluate intracellular protein expression of Syk (25) and ZAP-70 will distinguish between these possibilities. Finally, we cannot rule out that developmental stages in the hematopoietic lineage before the acquisition of a pre-TCR or a γδ TRC may be affected by the absence of Syk.

Further T cell development in Syk$^{-/-}$ *chimeras*

Since the lymphoid reconstitution in our *Syk*$^{-/-}$ → RAG2 chimeras was largely defective, the effects of Syk deficiency on later stages of T cell development was analyzed by comparing wt and *Syk*$^{-/-}$ FL-HSC chimeras in more permissive RAG2/γc mice. DP thymocytes, CD4, and CD8 SP thymocytes, γδ T cells, and NK-T cells were all reduced in *Syk*$^{-/-}$ chimeras, ranging from 1.5- to 3.5-fold less than in control chimeras (Fig. 1*A* and Table I). However, once past this developmental checkpoint, Syk deficiency appears to have no differential effect on the subsequent development of unique T cell subsets, consistent with a low expression of Syk in mature T cells.

Absolute numbers of peripheral T cell subsets were also reduced in *Syk*$^{-/-}$ FL-HSC → RAG2/γc chimeras as compared with wt chimeras (Fig. 3 and Table III). The overall reduction was on the order of 3- to 4-fold for splenic CD4, CD8, and γδ T cells and for splenic and hepatic NK-T cells. Nonredundant, cell-autonomous functions of Syk in early lymphoid development may explain the lower global cellularity in the periphery of *Syk*$^{-/-}$ chimeras. However, since the survival and expansion of peripheral αβ T cells requires TCR-MHC interactions (reviewed in Ref. 28), the inability of Syk-deficient T cells to maintain peripheral homeostasis would also be consistent with a role for Syk in the signal transduction pathways involved in this process, at least in those mature T cells that maintain a high expression of Syk (Table II and Ref. 2). Moreover, the lower cellularity in the periphery of *Syk*$^{-/-}$ chimeras may also result from different repertoires in *Syk*$^{-/-}$ T cell populations, as it has been shown that homeostatic control of peripheral T cells may be related to TCR specificity (29).

Intestinal γδ T cells develop in the absence of Syk

We analyzed the development of gut-associated lymphoid cells, a substantial fraction of which derive from an extrathymic pathway

Table III. *Lymphoid reconstitution of RAG2/γ_c and RAG2 mice injected with wt and* Syk$^{-/-}$ *FL cells*[a]

		Spleen (absolute numbers)					Intestine[b] (%)					
Host	Donor	CD4 (10^6)	CD8 (10^6)	γδ T (10^4)	NK T (10^4)	*n*	IEL/EC[c]	γδ T	αβ TI	CD4	CD8β	*n*
RAG2/$\gamma_c^{-/-}$	wt	11 ± 4.1	3.1 ± 1.3	25 ± 10	25 ± 14	5	14 ± 7	49 ± 14	12 ± 6	3.8 ± 3.6	27 ± 12	8
RAG2/$\gamma_c^{-/-}$	*Syk*$^{-/-}$	2.7 ± 0.6	1.3 ± 0.4	7 ± 4	6.5 ± 3	7	15 ± 9	21 ± 12	19 ± 10	7.5 ± 8	51 ± 23	8
RAG2$^{-/-}$	wt	8.5 ± 1.1	3.1 ± 0.5	29 ± 2.7	22 ± 0.6	3	10	42	25	2.5	22	2
RAG2$^{-/-}$	*Syk*$^{-/-}$	0.5 ± 0.6	0.1 ± 0.1	0.2 ± 0.3	1.7 ± 0.4	3	1.7	5	ND	ND	ND	2

[a] Spleens were explanted 8–12 wk after transfer, and cell suspension was enumerated. After calculating the percentage of lymphoid cells (about 65% of total in *wt* reconstituted spleens and 50% in *Syk*$^{-/-}$ reconstituted spleens) on the basis of their forward light scatter/side scatter profiles, the numbers of the indicated population were calculated.
[b] IELs were isolated 8–12 wk after transfer. Values are expressed as percentages of IELs out of epithelial cells. All IEL subsets are expressed as percentages of total IELs.
[c] EC, epithelial cells; TI, thymo-independent; ND, not determined.

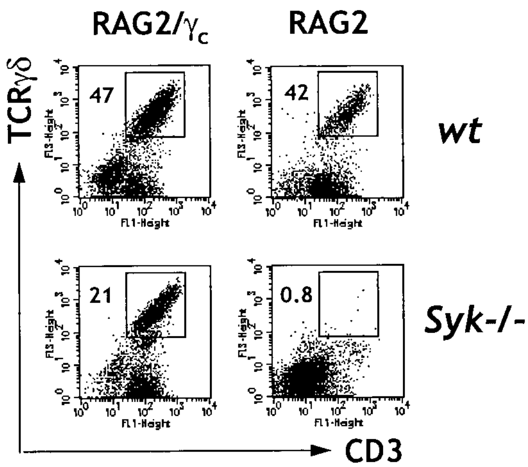

FIGURE 4. Development of *Syk*$^{-/-}$ $\gamma\delta$ IELs in RAG/γc chimeras but not in RAG2 chimeras. The intestines were explanted from the indicated mice 8–12 wk after transfer. Lymphoid cells were purified and cell suspensions were stained with mAbs specific for CD3 and TCR$\gamma\delta$. wt and *Syk*$^{-/-}$ refer to the genotype of the donor FL cells. Data are from one representative of two independent experiments.

(reviewed in Ref. 30). Previous studies have reported that the intestinal intraepithelial $\gamma\delta$ 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 $\rightarrow$ RAG2/γc chimeras, with an average of 14–15 IELs/100 epithelial cells. Almost half of the IELs were defined as $\gamma\delta$ T cells in both RAG2 and RAG/γc chimeras generated with wt FL-HSC, whereas $\gamma\delta$ T cells accounted for 21 $\pm$ 12% of the IELs in *Syk*$^{-/-}$ FL-HSC $\rightarrow$ RAG2/γc mice; the remaining cells in both sets of chimeras were $\alpha\beta$ T IELs (Table III). Thus, Syk deficiency results in only a 2-fold reduction in $\gamma\delta$ T IELs, and Syk is therefore not essential for development of this subset.

Our results are in striking contrast to the severe reduction in *Syk*$^{-/-}$ intestinal $\gamma\delta$ T IELs previously reported by Mallick-Wood et al. (12). It is important to note that those results were obtained by studying embryo aggregation chimeras. Under those conditions, the development of the two genetically different populations occurs under physiological conditions, as the aggregation event for embryogenesis is made before organogenesis (at the four- to eight-cell stage embryo (31). Although it would not be correct to directly compare the two experimental settings, it is clear that under our conditions, *Syk*$^{-/-}$ FL cells have a marked competitive disadvantage against host RAG2 elements, more severe than in RAG2/γc hosts, whereas control FL-HSC differentiate readily in both mice. Our results argue in favor of a competitive disadvantage of Syk-deficient lymphoid precursors against host elements. This hypothesis was confirmed in our setting: RAG2 mice reconstituted with wt FL-HSC generated a normal profile of T IEL subsets, whereas IEL development was severely compromised in RAG2 mice reconstituted with *Syk*$^{-/-}$ FL-HSC (Fig. 4 and Table III).

Mallick-Wood et al. (12) reported that DETCs were also virtually absent in *Syk*$^{-/-}$ $\leftrightarrow$ RAG2 allophenic chimeras. We could not test for DETCs, as hematopoietic chimeras generated by FL-HSC cannot develop DETCs at all because of asynchrony between the developmental stages of the donor fetal cells and the adult thymic environment of the host. However, a few viable *Syk*$^{-/-}$ mice do develop DETCs, although their numbers are reduced to 60% of control (6). Moreover, *Zap70*$^{-/-}$ mice develop normal numbers of DETCs (which are however morphologically abnormal (13), in keeping with the notions that neither Syk nor ZAP-70 are essential for DETC development.

Conclusion

The generation of experimental chimeras by irradiating mice and reconstituting them with hematopoietic stem cells has provided the opportunity to investigate the role of many gene products (including otherwise lethal mutations) in lymphoid development. However, the conclusions based on this approach must be interpreted with caution, especially when assigning an essential role to a given gene for the development of a given lymphoid subset. Our results show that certain mutations can engender a selective disadvantage to the developing hematopoieitc cells, resulting in their inability to effectively compete with host elements for developmental niches. Along these lines, recent reports from our laboratory (19) and those of Takeda et al. (32) have shown that HSC bearing a null mutation in the c-*kit* receptor for stem cell factor fail to give rise to T cells when injected into RAG2-deficient mice, whereas they generate the complete range of T cell subsets when injected into RAG2/γc mice. The results presented herein suggest a novel role for Syk in early T cell development and show that competition with residual host elements can strongly influence the outcome of hematopoietic reconstitution experiments, even in sublethally irradiated recipients.

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References

1. Bolen, J. B., and J. S. Brugge. 1997. Leukocyte protein tyrosine kinases: potential targets for drug discovery. *Annu. Rev. Immunol. 15:371.*
2. Chu, D. H., C. T. Morita, and A. Weiss. 1998. The Syk family of protein tyrosine kinases in T-cell activation and development. *Immunol. Rev. 165:167.*
3. Cooke, M. P., K. M. Abraham, K. A. Forbush, and R. M. Perlmutter. 1991. Regulation of T cell receptor signaling by a src family protein-tyrosine kinase (p59fyn). *Cell 65:281.*
4. van Oers, N. S., N. Killeen, and A. Weiss. 1996. Lck regulates the tyrosine phosphorylation of the T cell receptor subunits and ZAP-70 in murine thymocytes. *J. Exp. Med. 183:1053.*
5. Negishi, I., N. Motoyama, K. Nakayama, K. Nakayama, S. Senju, S. Hatakeyama, Q. Zhang, A. C. Chan, and D. Y. Loh. 1995. Essential role for ZAP-70 in both positive and negative selection of thymocytes. *Nature 376:435.*
6. Turner, M., P. J. Mee, P. S. Costello, O. Williams, A. A. Price, L. P. Duddy, M. T. Furlong, R. L. Geahlen, and V. L. Tybulewicz. 1995. Perinatal lethality and blocked B-cell development in mice lacking the tyrosine kinase Syk. *Nature 378:298.*
7. Cheng, A. M., B. Rowley, W. Pao, A. Hayday, J. B. Bolen, and T. Pawson. 1995. Syk tyrosine kinase required for mouse viability and B-cell development. *Nature 378:303.*
8. Turner, M., A. Gulbranson-Judge, M. E. Quinn, A. E. Walters, I. C. M. MacLennan, and V. Tybulewicz. 1997. Syk tyrosine kinase is required for the positive selection of immature B cells into the recirculating B cell pool. *J. Exp. Med. 186:2013.*
9. Colucci, F., M. Turner, E. Schweighoffer, D. Guy-Grand, V. Di Bartolo, M. Salcedo, V. L. Tybulewicz, and J .P. Di Santo. 1999. Redundant role of the syk protein tyrosine kinase in mouse NK cell differentiation. *J. Immunol. 163: 1769.*
10. Chan, A. C., N. S. van Oers, A. Tran, L. Turka, C. L. Law, J. C. Ryan, E. A. Clark, and A. Weiss. 1994. Differential expression of ZAP-70 and Syk protein tyrosine kinases, and the role of this family of protein tyrosine kinases in TCR signaling. *J. Immunol. 152:4758.*
11. Haas, W., P. Pereira, and S. Tonegawa. 1993. Gamma/delta cells. *Annu. Rev. Immunol. 11:637.*
12. Mallick-Wood, C. A., W. Pao, A. C. Cheng, J. M. Lewis, S. Kulkarni, J. B. Bolen, B. Rowley, R. T. Tigelaar, T. Pawson, and A. C. Hayday. 1996. Disruption of epithelial gd T cell repertoires by mutation of the Syk tyrosine kinase. *Proc. Natl. Acad. Sci. USA 93:9704.*

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.*
15. Colucci, F., C. Soudais, E. Rosmaraki, L. Vanes, V. L. J. Tybulewicz, and J. P. Di Santo. 1999. Dissecting NK cell development using a novel alymphoid mouse model: investigating the role of c-abl proto-oncogene in murine NK differentiation. *J. Immunol. 162:2761.*
16. Shinkai, Y., G. Rathbun, K. P. Lam, E. M. Oltz, V. Stewart, M. Mendelsohn, J. Charron, M. Datta, F. Young, A. M. Stall, et al. 1992. RAG-2-deficient mice lack mature lymphocytes owing to inability to initiate V(D)J rearrangement. *Cell 68:855.*
17. Guy-Grand, D., J. B. Cuenod, S. M. Malassis, F. Selz, and P. Vassalli. 1996. Complexity of the mouse gut T cell immune system: identification of two distinct natural killer T cell intraepithelial lineages. *Eur. J. Immunol. 26:2248.*
18. Wilson, A., and H. R. MacDonald. 1995. Expression of genes encoding the pre-TCR and CD3 complex during thymus development. *Int. Immunol. 7:1659.*
19. Colucci, F., and J. P. Di Santo. 2000. The receptor Tyrosine kinase c-*kit* provides a critical signal for survival, expansion and maturation of mouse natural killer cells. *Blood 95:984.*
20. Kiefer, F., J. Brumell, N. Al-Alawi, S. Latour, A. Cheng, A. Veillette, S. Grinstein, and T. Pawson. 1998. The Syk protein tyrosine kinase is essential for Fc γ receptor signaling in macrophages and neutrophils. *Mol. Cell. Biol. 18:4209.*
21. Godfrey, D. I., J. Kennedy, T. Suda, and A. Zlotnik. 1993. A developmental pathway involving four phenotypically and functionally distinct subsets of $CD3^-CD4^-CD8^-$ triple-negative adult mouse thymocytes defined by CD44 and CD25 expression. *J. Immunol. 150:4244.*
22. Godfrey, D. I., J. Kennedy, P. Mombaerts, S. Tonegawa, and A. Zlotnik. 1994. Onset of TCR-β gene rearrangement and role of TCR-β expression during $CD3^-CD4^-CD8^-$ thymocyte differentiation. *J. Immunol. 152:4783.*
23. Capone, M., R. D. J. Hockett, and A. Zlotnik. 1998. Kinetics of T cell receptor β, γ, and δ rearrangements during adult thymic development: T cell receptor rearrangements are present in $CD44^+CD25^+$ pro-T thymocytes. *Proc. Natl. Acad. Sci. USA 95:12522.*
24. Cheng, A. M., I. Negishi, S. J. Anderson, A. C. Chan, J. Bolen, D. Y. Loh, and T. Pawson. 1997. The Syk and ZAP-70 SH2-containing tyrosine kinases are implicated in pre-T cell receptor signaling. *Proc. Natl. Acad. Sci. USA 94:9797.*
25. Chu, D. H., N. S. van Oers, M. Malissen, J. Harris, M. Elder, and A. Weiss. 1999. Pre-T cell receptor signals are responsible for the down-regulation of Syk protein tyrosine kinase expression. *J. Immunol. 163:2610.*
26. Kolanus, W., C. Romeo, and B. Seed. 1993. T cell activation by clustered tyrosine kinases. *Cell 74:171.*
27. Chu, D. H., H. Spits, J. F. Peyron, R. B. Rowley, J. B. Bolen, and A. Weiss. 1996. The Syk protein tyrosine kinase can function independently of CD45 or Lck in T cell antigen receptor signaling. *EMBO J. 15:6251.*
28. Freitas, A. A., and B. Rocha. 1999. Peripheral T cell survival. *Curr. Opin. Immunol. 11:152.*
29. Ernst, B., D. S. Lee, J. M. Chang, J. Sprent, and C. D. Surh. 1999. The peptide ligands mediating positive selection in the thymus control T cell survival and homeostatic proliferation in the periphery. *Immunity 11:173.*
30. Rocha, B., D. Guy-Grand, and P. Vassalli. 1995. Extrathymic T cell differentiation. *Curr. Opin. Immunol. 7:235.*
31. McLaren, A., and P. Bowman. 1969. Mouse chimeras derived from fusion of embryos differing by nine genetic factors. *Nature 224:238.*
32. Takeda, S., T. Shimizu, and H. R. Rodewald. 1997. Interactions between c-*kit* and stem cell factor are not required for B-cell development in vivo. *Blood 89:518.*