A New Look at Syk in αβ and γδ 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

**References**

This article cites 32 articles, 17 of which you can access for free at:

http://www.jimmunol.org/content/164/10/5140.full#ref-list-1

**Subscriptions**

Information about subscribing to *The Journal of Immunology* is online at:

http://jimmunol.org/subscriptions

**Permissions**

Submit copyright permission requests at:

http://www.aai.org/ji/copyright.html

**Email Alerts**

Receive free email-alerts when new articles cite this article. Sign up at:

http://jimmunol.org/cgi/alerts/etoc
A New Look at Syk in αβ and γδ T Cell Development Using Chimeric Mice with a Low Competitive Hematopoietic Environment

Francesco Colucci,2* Delphine Guy-Grand, Anne Wilson, † Martin Turner, Edina Schweighoffer,† Victor L. J. Tybulewicz,vl 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., γδ 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<sup>−/−</sup> 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 CD4<sup>+</sup>CD8<sup>−</sup> stage. According to previous reports, development of intestinal γδ T cells was arrested in Syk<sup>−/−</sup> →RAG2<sup>−/−</sup> chimeras. In striking contrast, when hosts were the newly established alymphoid RAG2 × common cytokine receptor γ-chain (RAG2γc) mice, Syk<sup>−/−</sup> chimeras developed intestinal γδ T cells as well as other T cell subsets (including αβ T cells, NK1.1<sup>+</sup> αβ T cells, and splenic and thymic γδ 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 γδ T cell lineage to develop. The Journal of Immunology, 2000, 164: 5140–5145.

The Syk and ZAP-70 PTKs play unique roles in B cells and most T cell subsets, the reduction being likely to relate to abnormal 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<sup>−/−</sup>) are viable (5), Syk<sup>−/−</sup> 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, αβ 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<sup>+</sup>CD8<sup>+</sup> double-positive (DP) stage, whereas B cell development in Zap70<sup>−/−</sup> 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 αβ 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 γδ 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 γδ T cells that home preferentially to epithelial tissues include the skin dendritic epidermal T cells (DETs) 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 γδ T cell subsets, the reduction being likely to relate to abnormal

---

1 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, Epalinges, Switzerland; Babraham Institute, Babraham, Cambridge, United Kingdom; National Institute for Medical Research, London, United Kingdom

Received for publication December 13, 1999. Accepted for publication March 3, 2000.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

© 2000 by The American Association of Immunologists

Copyright © 2000 by The American Association of Immunologists

0022-1767/00/$02.00

0822-1767/00/502.00
development of precursors during fetal life (6). Allophosph chimeras generated by aggregating Syk<sup>−/−</sup> and recombination-activating gene (RAG) 2<sup>−/−</sup> 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<sup>−/−</sup> 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 RAG<sup>2−/−</sup> 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 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<sup>−/−</sup> HSCs which do not develop in Syk<sup>−/−</sup> allophosph chimeras in the RAG<sup>2−/−</sup> 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<sup>−/−</sup> HSCs. We have developed a novel allopheophenoid mouse strain by combining RAG2 and common cytokine receptor γ-chain (γc) mutations (RAG2/γc<sup>−/−</sup> mice (15)). The absence of lymphoid progenitors in RAG2/γc<sup>−/−</sup> 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<sup>−/−</sup> (RAG2; Ref. 16) and RAG2<sup>−/−</sup>/γc<sup>−/−</sup> (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<sup>+/+</sup> or Syk<sup>−/−</sup>) embryos as described (10). The absence of lymphoid progenitors in RAG2/γc<sup>−/−</sup> 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.

#### 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-2Kb, TCROβ, TCRγδ, 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<sup>+</sup> CD8<sup>−</sup> thymocytes were prepared by complement-mediated depletion with anti-CD4 and anti-CD8 Abs followed by Dynabead (Dynal, Compiegne, France) depletion with anti-CD3 Abs to remove all mature cells. CD4<sup>+</sup> CD8<sup>−</sup> CD25<sup>−</sup> CD44<sup>−</sup> CD25<sup>−</sup> CD4<sup>+</sup> CD8<sup>+</sup> thymic subsets, CD3<sup>+</sup> lymph node (LN) T cells or B220<sup>−</sup> LN B cells, CD4<sup>+</sup> CD8<sup>−</sup> (DP), CD4<sup>+</sup> CD8<sup>+</sup> immature single positive (SP), and CD4<sup>+</sup> CD8<sup>+</sup> CD3<sup>+</sup> (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, protease K treatment, and acetylation, specific mRNA transcripts were detected by hybridization with<sup>35</sup>S-UTP labeled RNA probes as follows: for ZAP-70, a 700-bp EcoRI/FseI fragment was cloned into pSP73; for Syk, a 700-bp XbaI/EcoRI fragment was cloned into pBluescript SK<sup>−</sup>. 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 (0.5 Gy) RAG2 vs RAG2/γc mice (H-2<sup>b</sup>) reconstituted with day 16 fetal liver HSC (FL-HSC) from wt or Syk-deficient embryos (H-2<sup>b</sup>). 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 quantititated the numbers of B and T cells (including αβ, γδ, and NK1.1<sup>+</sup> 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. 1A), 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<sup>−/−</sup>→ RAG2/γc chimeras than in Syk<sup>−/−</sup>→
RAG2 chimeras (Table I and Fig. 1A). 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. 1A) that were host derived (negative for H-2^d; Fig. 1B). 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. 1A). 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^−TCRαβ^−TCRγδ^−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^+ (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 γδ 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 → 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...
isolated by FACS sorting and cells were analyzed for Syk and ZAP-70 expression. The transitional block of Syk-deficient CD44
of a pre-TCR or a development. The transitional block of Syk-deficient CD44
2
or CD3, NK1.1, and B220. Host-derived cells (H-2\textsuperscript{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\textsuperscript{-/-} refer to the genotype of the donor FL cells. Percentages of boxed populations are indicated. Data are from one representative of five independent experiments.

Further T cell development in Syk\textsuperscript{+/+} chimeras

Since the lymphoid reconstitution in our Syk\textsuperscript{+/+} \rightarrow RAG2 chimeras was largely defective, the effects of Syk deficiency on later stages of T cell development was analyzed by comparing wt and Syk\textsuperscript{+/+} 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\textsuperscript{+/+} chimeras, ranging from 1.5- to 3.5-fold less than in control chimeras (Fig. 1A 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\textsuperscript{+/+} FL-HSC \rightarrow 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\textsuperscript{+/+} 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\textsuperscript{+/+} chimeras may also result from different repertoires in Syk\textsuperscript{+/+} 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 II. Expression of Syk and ZAP-70 in early thymocytes by in situ hybridization\textsuperscript{a}

<table>
<thead>
<tr>
<th>Subset</th>
<th>Syk</th>
<th>ZAP-70</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD44\textsuperscript{CD25}\textsuperscript{-}</td>
<td>74 ± 10</td>
<td>18 ± 4</td>
</tr>
<tr>
<td>CD44\textsuperscript{CD25}\textsuperscript{+}</td>
<td>30 ± 4</td>
<td>18 ± 5</td>
</tr>
<tr>
<td>CD44\textsuperscript{CD25}\textsuperscript{+}</td>
<td>22 ± 8</td>
<td>29 ± 5</td>
</tr>
<tr>
<td>CD44\textsuperscript{CD25}\textsuperscript{-}</td>
<td>40 ± 5</td>
<td>37 ± 10</td>
</tr>
<tr>
<td>ISP\textsuperscript{a}</td>
<td>6 ± 3</td>
<td>37 ± 10</td>
</tr>
<tr>
<td>DP\textsuperscript{a}</td>
<td>4 ± 3</td>
<td>28 ± 10</td>
</tr>
<tr>
<td>SP CD8\textsuperscript{a}</td>
<td>4 ± 3</td>
<td>25 ± 10</td>
</tr>
<tr>
<td>LN T</td>
<td>7 ± 4</td>
<td>29 ± 8</td>
</tr>
<tr>
<td>LN B</td>
<td>79 ± 2</td>
<td>5 ± 2</td>
</tr>
<tr>
<td>γδ T</td>
<td>4 ± 2</td>
<td>68 ± 2</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Thymi and LN were explanted from adult B6 mice. The indicated subsets were isolated by FACS sorting and 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. 

### Table III. Lymphoid reconstitution of RAG2/γc and RAG2 mice injected with wt and Syk\textsuperscript{+/+} FL cells\textsuperscript{a}

<table>
<thead>
<tr>
<th>Host</th>
<th>Donor</th>
<th>Spleen (×10\textsuperscript{6})</th>
<th>Intestine\textsuperscript{b} (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>CD4 (10\textsuperscript{6})</td>
<td>CD8 (10\textsuperscript{6})</td>
</tr>
<tr>
<td>RAG2\textsuperscript{+/+}</td>
<td>wt</td>
<td>11 ± 4</td>
<td>3.1 ± 1.3</td>
</tr>
<tr>
<td>RAG2\textsuperscript{+/+}</td>
<td>Syk\textsuperscript{-/-}</td>
<td>2.7 ± 0.6</td>
<td>1.3 ± 0.4</td>
</tr>
<tr>
<td>RAG2\textsuperscript{-/-}</td>
<td>wt</td>
<td>85 ± 1.1</td>
<td>3.1 ± 0.5</td>
</tr>
<tr>
<td>RAG2\textsuperscript{-/-}</td>
<td>Syk\textsuperscript{-/-}</td>
<td>0.5 ± 0.6</td>
<td>0.1 ± 0.1</td>
</tr>
</tbody>
</table>

\textsuperscript{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\textsuperscript{-/-} reconstituted spleens) on the basis of their forward light scatter/side scatter profiles, the numbers of the indicated population were calculated.

\textsuperscript{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.

\textsuperscript{c} EC, epithelial cells; TI, thymo-independent; ND, not determined.
(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/\( \gamma c \) mice injected with FL-HSC (Fig. 4). Although it is difficult to accurately quantify 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/\( \gamma c \) 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 RAG2/\( \gamma c \) chimeras generated with wt FL-HSC, whereas \( \gamma \delta \) T cells accounted for 21 ± 12% of the IELs in Syk\(^{-/-} \) FL-HSC → RAG2/\( \gamma c \) 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/\( \gamma c \) 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\(^{-/-} \) ↔ 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 hematopoietic 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/\( \gamma c \) 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.

**Acknowledgments**

We appreciate the excellent technical assistance of Michelle Malassis.

**References**


