In Vivo Administration of the Recombinant IL-7/Hepatocyte Growth Factor β Hybrid Cytokine Efficiently Restores Thymopoiesis and Naive T Cell Generation in Lethally Irradiated Mice after Syngeneic Bone Marrow Transplantation

Jingjun Jin, Irving Goldschneider and Laijun Lai

*J Immunol* published online 5 January 2011
http://www.jimmunol.org/content/early/2011/01/05/jimmunol.1001238

Supplementary Material
http://www.jimmunol.org/content/suppl/2011/01/05/jimmunol.1001238.DC1

Why *The JI*? Submit online.
- *Rapid Reviews!* 30 days* from submission to initial decision
- *No Triage!* Every submission reviewed by practicing scientists
- *Fast Publication!* 4 weeks from acceptance to publication

*average

Subscription
Information about subscribing to *The Journal of Immunology* is online at:
http://jimmunol.org/subscription

Permissions
Submit copyright permission requests at:
http://www.aai.org/About/Publications/JI/copyright.html

Email Alerts
Receive free email-alerts when new articles cite this article. Sign up at:
http://jimmunol.org/alerts

*The Journal of Immunology* is published twice each month by
The American Association of Immunologists, Inc.,
1451 Rockville Pike, Suite 650, Rockville, MD 20852
Copyright © 2011 by The American Association of Immunologists, Inc. All rights reserved.
Print ISSN: 0022-1767 Online ISSN: 1550-6606.
In Vivo Administration of the Recombinant IL-7/Hepatocyte Growth Factor β Hybrid Cytokine Efficiently Restores Thymopoiesis and Naive T Cell Generation in Lethally Irradiated Mice after Syngeneic Bone Marrow Transplantation

Jingjun Jin,* Irving Goldschneider,* and Laijun Lai*†

Bone marrow transplantation (BMT) is often followed by a prolonged period of T cell deficiency. Therefore, the enhancement of T cell reconstitution is an important clinical goal. We have identified a novel hybrid cytokine containing IL-7 and the β-chain of hepatocyte growth factor (HGF) in the supernatant of cultured mouse BM stromal cells. We have cloned and expressed the IL-7/ HGFβ gene to produce a single-chain rIL-7/HGFβ protein that stimulates the in vitro proliferation of thymocytes, early B-lineage cell, and day 12 spleen CFUs. In this study, we show that, following syngenic BMT, the in vivo administration of rIL-7/HGFβ supports the rapid and complete regeneration of the thymus and efficiently reconstitutes the pool of naive T cells having a normally diverse TCR repertoire. The rIL-7/HGF hybrid cytokine was significantly more effective quantitatively than was rIL-7 and differed qualitatively in its ability to cross-link c-Met and IL-7Rα and to stimulate the expansion of early thymocyte progenitors and thymic epithelial cells. It also supports the maturation and homeostatic expansion of peripheral T cells. Consequently, the in vivo administration of rIL-7/HGFβ may offer a new approach to preventing and/or correcting post-BMT T cell immune deficiency.

The Journal of Immunology, 2011, 186: 000–000.

Department of Immunology, School of Medicine, University of Connecticut Health Center, Farmington, CT 06030; and University of Connecticut Stem Cell Institute, School of Medicine, University of Connecticut Health Center, Farmington, CT 06030

Received for publication April 16, 2010. Accepted for publication November 29, 2010.

Address correspondence and reprint requests to Dr. Laijun Lai, Department of Immunology, University of Connecticut Health Center, 263 Farmington Avenue, Farmington, CT 06030. E-mail address: lla@neuron.uchc.edu

The online version of this article contains supplemental material.

Abbreviations used in this article: B6, C57BL/6; BMT, bone marrow transplantation; DN, double-negative; DP, double-positive; ETP, early thymocyte progenitor; GVHD, graft-versus-host disease; HGF, hepatocyte growth factor; MHC II, MHC class II; RTE, recent thymic emigrant; SP, single-positive; TCD, T cell-deleted; TEC, thymic epithelial cell; WT, wild-type.

Copyright © 2011 by The American Association of Immunologists, Inc. 0022-1767/11/S16.00

*Department of Immunology, School of Medicine, University of Connecticut Health Center, Farmington, CT 06030; and University of Connecticut Stem Cell Institute, School of Medicine, University of Connecticut Health Center, Farmington, CT 06030

Received for publication April 16, 2010. Accepted for publication November 29, 2010.

Address correspondence and reprint requests to Dr. Laijun Lai, Department of Immunology, University of Connecticut Health Center, 263 Farmington Avenue, Farmington, CT 06030. E-mail address: lla@neuron.uchc.edu

The online version of this article contains supplemental material.

Abbreviations used in this article: B6, C57BL/6; BMT, bone marrow transplantation; DN, double-negative; DP, double-positive; ETP, early thymocyte progenitor; GVHD, graft-versus-host disease; HGF, hepatocyte growth factor; MHC II, MHC class II; RTE, recent thymic emigrant; SP, single-positive; TCD, T cell-deleted; TEC, thymic epithelial cell; WT, wild-type.

Copyright © 2011 by The American Association of Immunologists, Inc. 0022-1767/11/S16.00

*Department of Immunology, School of Medicine, University of Connecticut Health Center, Farmington, CT 06030; and University of Connecticut Stem Cell Institute, School of Medicine, University of Connecticut Health Center, Farmington, CT 06030

Received for publication April 16, 2010. Accepted for publication November 29, 2010.

Address correspondence and reprint requests to Dr. Laijun Lai, Department of Immunology, University of Connecticut Health Center, 263 Farmington Avenue, Farmington, CT 06030. E-mail address: lla@neuron.uchc.edu

The online version of this article contains supplemental material.

Abbreviations used in this article: B6, C57BL/6; BMT, bone marrow transplantation; DN, double-negative; DP, double-positive; ETP, early thymocyte progenitor; GVHD, graft-versus-host disease; HGF, hepatocyte growth factor; MHC II, MHC class II; RTE, recent thymic emigrant; SP, single-positive; TCD, T cell-deleted; TEC, thymic epithelial cell; WT, wild-type.

Copyright © 2011 by The American Association of Immunologists, Inc. 0022-1767/11/S16.00

*Department of Immunology, School of Medicine, University of Connecticut Health Center, Farmington, CT 06030; and University of Connecticut Stem Cell Institute, School of Medicine, University of Connecticut Health Center, Farmington, CT 06030

Received for publication April 16, 2010. Accepted for publication November 29, 2010.

Address correspondence and reprint requests to Dr. Laijun Lai, Department of Immunology, University of Connecticut Health Center, 263 Farmington Avenue, Farmington, CT 06030. E-mail address: lla@neuron.uchc.edu

The online version of this article contains supplemental material.

Abbreviations used in this article: B6, C57BL/6; BMT, bone marrow transplantation; DN, double-negative; DP, double-positive; ETP, early thymocyte progenitor; GVHD, graft-versus-host disease; HGF, hepatocyte growth factor; MHC II, MHC class II; RTE, recent thymic emigrant; SP, single-positive; TCD, T cell-deleted; TEC, thymic epithelial cell; WT, wild-type.

Copyright © 2011 by The American Association of Immunologists, Inc. 0022-1767/11/S16.00

*Department of Immunology, School of Medicine, University of Connecticut Health Center, Farmington, CT 06030; and University of Connecticut Stem Cell Institute, School of Medicine, University of Connecticut Health Center, Farmington, CT 06030

Received for publication April 16, 2010. Accepted for publication November 29, 2010.

Address correspondence and reprint requests to Dr. Laijun Lai, Department of Immunology, University of Connecticut Health Center, 263 Farmington Avenue, Farmington, CT 06030. E-mail address: lla@neuron.uchc.edu

The online version of this article contains supplemental material.

Abbreviations used in this article: B6, C57BL/6; BMT, bone marrow transplantation; DN, double-negative; DP, double-positive; ETP, early thymocyte progenitor; GVHD, graft-versus-host disease; HGF, hepatocyte growth factor; MHC II, MHC class II; RTE, recent thymic emigrant; SP, single-positive; TCD, T cell-deleted; TEC, thymic epithelial cell; WT, wild-type.

Copyright © 2011 by The American Association of Immunologists, Inc. 0022-1767/11/S16.00

*Department of Immunology, School of Medicine, University of Connecticut Health Center, Farmington, CT 06030; and University of Connecticut Stem Cell Institute, School of Medicine, University of Connecticut Health Center, Farmington, CT 06030

Received for publication April 16, 2010. Accepted for publication November 29, 2010.

Address correspondence and reprint requests to Dr. Laijun Lai, Department of Immunology, University of Connecticut Health Center, 263 Farmington Avenue, Farmington, CT 06030. E-mail address: lla@neuron.uchc.edu

The online version of this article contains supplemental material.

Abbreviations used in this article: B6, C57BL/6; BMT, bone marrow transplantation; DN, double-negative; DP, double-positive; ETP, early thymocyte progenitor; GVHD, graft-versus-host disease; HGF, hepatocyte growth factor; MHC II, MHC class II; RTE, recent thymic emigrant; SP, single-positive; TCD, T cell-deleted; TEC, thymic epithelial cell; WT, wild-type.

Copyright © 2011 by The American Association of Immunologists, Inc. 0022-1767/11/S16.00

*Department of Immunology, School of Medicine, University of Connecticut Health Center, Farmington, CT 06030; and University of Connecticut Stem Cell Institute, School of Medicine, University of Connecticut Health Center, Farmington, CT 06030

Received for publication April 16, 2010. Accepted for publication November 29, 2010.

Address correspondence and reprint requests to Dr. Laijun Lai, Department of Immunology, University of Connecticut Health Center, 263 Farmington Avenue, Farmington, CT 06030. E-mail address: lla@neuron.uchc.edu

The online version of this article contains supplemental material.

Abbreviations used in this article: B6, C57BL/6; BMT, bone marrow transplantation; DN, double-negative; DP, double-positive; ETP, early thymocyte progenitor; GVHD, graft-versus-host disease; HGF, hepatocyte growth factor; MHC II, MHC class II; RTE, recent thymic emigrant; SP, single-positive; TCD, T cell-deleted; TEC, thymic epithelial cell; WT, wild-type.

Copyright © 2011 by The American Association of Immunologists, Inc. 0022-1767/11/S16.00
Carlsbad, CA) supplemented with sodium bicarbonate (2 mg/ml) and 1% HEPES. T cell-deleted (TCD)-BM was prepared by incubating the BM cell suspensions with saturating amounts of rat anti-CD4, anti-CD8, and anti-Thy1.2 mAbs, developing with goat anti-rat IgG magnetic beads (Miltenyi Biotec, Auburn, CA), and running through an immunomagnetic depletion column, as described (22). Recipient B6 mice received 1000 cGy total

FIGURE 1. Expression of c-Met and IL-7Rα by cells in the normal thymus. Flow cytometric analysis of thymocyte subsets (DN, DP, CD4 SP, CD8 SP) for the expression of IL-7Rα (A–D) and c-Met (E–H) and of ETPs (Lin− IL-7Rα− c-Kit−CD44−CD25− ) and TECs (CD45+EpCAM+MHC+) for the expression of c-Met (I, K) and IL-7Rα (J, L). Solid lines represent fluorescence profiles for the indicated receptors, and gray filled lines represent the corresponding isotype control. Representative profiles from one of four mice.

FIGURE 2. Effect of rIL-7/HGFβ administration on the numbers of donor-origin thymocytes after BMT. Lethally irradiated mice (B6) were injected i.v. with 2 × 10^6 congenic B6 Ly5.1 TCD-BM cells and i.p. with graded doses of rIL-7/HGFβ, rIL-7, rHGFβ, or PBS at 2-d intervals between days 1 and 26 after BMT. Thymic cellularity was analyzed on day 30 after BMT. A, Dose-response curves for equimolar amounts of rIL-7/HGFβ or rIL-7 relative to PBS-injected controls (horizontal line). B, Responses of the indicated thymocyte subsets to optimal equimolar amounts of rIL-7/HGFβ (15 μg), rIL-7 (5 μg), and/or rHGFβ (10 μg). *p < 0.05 compared with PBS-treated mice; **p < 0.05 compared with rIL-7 and/or rHGFβ-treated mice; ***p < 0.05 compared with normal WT control. C, Percent inhibition of rIL-7/HGFβ (15 μg) stimulation of the indicated thymocyte subsets by the administration of anti-c-Met and/or IL-7Rα Abs. Isotype controls did not significantly inhibit the stimulatory effect of rIL-7/HGFβ (data not shown). The data are representative of three independent experiments with three to six mice per group. *p < 0.05 compared with DP thymocytes.
Flow cytometric analysis

TECs were isolated by flow cytometry using the protocol of Gray et al. (25). Single-cell suspensions of thymocytes and splenic cells were stained with combinations of the following fluorochrome-conjugated Abs, as described (8): CD4, CD8, CD25, CD44, CD62L, CD117, CD127, BP-1, CD45, EpCAM, I-Ab, and c-Met (Biolegend, San Diego, CA; BD Biosciences). ETPs were identified by phenotypic analysis (Lin−c-Kit+IL-7Ra−CD44+CD25+) as described (26–29). An Ab mixture, composed of Abs against TCR Vβ families was purchased from BD Biosciences. ETPs were identified by flow cytometry. ETPs were differentiated into Lin−c-Kit−CD44−CD25 cells. The cells were analyzed on an FACScalibur instrument (BD Biosciences) and CellQuest acquisition software. Data analysis was done using FlowJo software (Ashland, OR).

FIGURE 3. Effect of rIL-7/HGFβ administration on the numbers of donor-origin ETPs after BMT. A, Lethally irradiated mice were transplanted with TCD-BM from congenic mice and treated with optimal doses of cytokines as in Fig. 2B. One month after BMT, donor-origin ETPs (Lin−c-Kit−IL-7Ra−CD44−CD25−) were quantified by flow cytometry. B, Percent inhibition of rIL-7/HGFβ (15 μg) stimulation by the administration of anti-c-Met and/or IL-7Rα Abs. Isotype controls did not significantly inhibit the stimulatory effect of rIL-7/HGFβ (data not shown). The data are representative of three independent experiments with three to six mice per group. *p < 0.05 compared with PBS-treated mice; **p < 0.05 compared with rIL-7 and/or rHGFβ-treated mice.

Immunoprecipitation of IL-7Ra

Purified CD4−CD8−CD90−CD44−CD11b−CD11c−NK1.1−TCRγδ−Medullary ETPs (Lin−c-Kit+IL-7Ra+CD44+CD25+) were immunoprecipitated with Ab against IL-7Ra and protein G-agarose bead slurry as described (8). The samples were analyzed on an Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA). The samples were analyzed on a genetic analyzer (ABI3730; Applied Biosystems) using GeneMarker software. The data are representative of three independent experiments with three to six mice per group. *p < 0.05 compared with rIL-7 and/or rHGFβ-treated mice; **p < 0.05 compared with rIL-7 and/or rHGFβ-treated mice.

Flow cytometry

Analysis was done using FlowJo software (Ashland, OR).
Results

Expression of c-Met and the IL-7R by thymocyte subsets, ETPs, and TECs

Similar to other reports, we have observed that the IL-7Rα-chain is expressed by most cells in the CD4+CD8+ DN and CD4+ or CD8+ single-positive (SP) thymocyte subsets, but not in the CD4+CD8+ double-positive (DP) subset (Fig. 1A–D). In contrast, c-Met is expressed by all thymocyte subsets, with DP thymocytes having the highest expression (Fig. 1E–H). c-Met, but not IL-7R, was also expressed by the ETPs (Lin–IL-7Rα–c-Kit+CD45+EpCAM+MHC+) (Fig. 1K, L) and by the TECs (CD45+EpCAM+MHC+) (Fig. 1K, L), the major components of the thymic microenvironment (34, 35).

rIL-7/HGFβ efficiently supports the regeneration of thymocytes in BMT mice

To determine whether rIL-7/HGFβ affects thymopoiesis following BMT, lethally irradiated C57BL/6 mice were injected i.v. with TCD-BM from B6 Ly 5.1 congenic donors and i.p. with graded doses of rIL-7/HGFβ (1.25, 2.5, 5, 10, 15, and 30 μg/injection) or control vehicle (PBS) at 2-d intervals between days 1 and 26. The numbers of donor-origin thymocytes (>86% of total) were analyzed on day 30. As shown in Fig. 2A, roughly parallel dose-response curves were observed between rIL-7/HGFβ and rIL-7 when the results were plotted as a function of equivalent m.w. (~3:1). However, the amplitude of the responses differed such that, at optimal equimolar doses (~1.5 times more effective at enhancing thymic cellularity than was rIL-7. This was true for each of the four major subsets of thymocytes, in which optimal doses of rIL-7 restored thymopoiesis to normal levels and rIL-7/HGFβ to supranormal levels, respectively (p < 0.05) (Fig. 2B). However, at suboptimal doses, rIL-7 (2 μg) increased the number of DN thymocytes only, whereas rIL-7/HGFβ (6 μg) increased the numbers of all thymocyte subsets (Supplemental Fig. 1).

The results also showed that rHGFβ, alone or mixed with rIL-7, had no detectable effects on thymocyte development. Nonetheless, as shown in Fig. 2C, Ab to c-Met inhibited the in vivo stimulatory effects of rIL-7/HGFβ on total donor-origin thymocytes by ~50%. This suggests that HGFβ attains thymocyte stimulatory activity when incorporated into the rIL-7/HGFβ hybrid cytokine. Interestingly, anti-c-Met was significantly more effective at inhibiting the stimulation of DN than DP thymocytes (~90% versus ~50%; p < 0.05), even though the DP subset expressed much higher levels of c-Met (Fig. 1E, 1F). This may be related to the failure of DP thymocytes to express the IL-7Rα-chain (Fig. 1B), as rIL-7/HGFβ cross-links c-Met and IL-7Rα on the surface of DN thymocytes (see below).

In this regard, anti–IL-7Rα Ab inhibited the in vivo stimulatory effects of rIL-7/HGFβ on total, DN, and SP thymocytes as efficiently as did anti-c-Met Ab (Fig. 2C). Somewhat surprisingly, anti–IL-7Rα also inhibited the generation of DP thymocytes, presumably secondary to its inhibitory effect on the stimulation of DN thymocytes. This may help to explain why a mixture of Abs to c-Met and IL-7Rα was more effective than either Ab alone at inhibiting the stimulation of DP thymocytes (~75% versus ~50%; p < 0.05). In addition, anti–IL-7Rα Ab had a significantly greater inhibitory effect on CD8 SP than CD4 SP thymocytes (Fig. 2C), even though both expressed comparable levels of IL-7Rα (Fig. 1C, 1D). This is consistent with the report of Brugnera et al. (36) that IL-7 stimulation is essential for the development of CD8 SP but not CD4 SP thymocytes from intermediate (CD4+CD8+) thymocytes. Interestingly, similar results were obtained with anti–c-Met Ab, suggesting as possibilities that the stimulation of c-Met also may be essential for CD8 SP but not CD4 SP development or that the rIL-7/HGFβ hybrid cytokine may have different binding patterns on CD8 SP and CD4 SP thymocytes.

**FIGURE 5.** Effect of rIL-7/HGFβ administration on the numbers of total and naive T cells in the spleen after BMT. Lethally irradiated mice were transplanted with TCD-BM from congenic mice and treated with optimal doses of cytokines as in Fig. 2B. One month after BMT, the numbers of total donor-origin CD4+ T cells, donor-origin naive CD4+CD8− T cells, donor-origin naive CD4+CD8− T cells, and total host-origin CD4− and CD8− splenic T cells were evaluated by flow cytometry. The data are representative of three independent experiments with three to six mice per group. *p < 0.05 compared with PBS-treated mice; **p < 0.05 compared with rIL-7 and/or rHGFβ-treated mice.
rIL-7/HGFβ but not rIL-7, increases the number of ETPs in BMT mice

ETPs (Lin⁺ IL-7Rα⁻ c-Kit⁺) constitute a minor subset of DN1 (CD44⁺CD25⁻) thymocytes that are thought to represent canonical thymocyte progenitors (26–29). We therefore determined whether the number of ETPs in the BMT recipients was altered by cytokine treatment. As shown in Fig. 3A, optimal doses of rIL-7 (5 μg) alone or mixed with rHGFβ (10 μg) were not significantly more effective than PBS at increasing the numbers of donor-origin ETPs (p > 0.05). In contrast, optimal doses of rIL-7/HGFβ (15 μg) increased the number of ETPs by ∼9-fold over those in the PBS-treated mice and ∼2-fold over those in normal wild-type (WT) controls. A suboptimal dose of rIL-7/HGFβ (6 μg) also significantly increased the number of ETPs (Supplemental Fig. 2). Also, as shown in Fig. 3B, the stimulatory effect of rIL-7/HGFβ was significantly inhibited by anti-c-Met. However, unlike its effects on most DN thymocytes (Fig. 2C), anti–IL-7Rα did not inhibit the stimulation of ETPs (Fig. 3B), presumably because ETPs do not express the IL-7Rα-chain (Fig. 1J).

Next, we determined whether cytokine treatment could prevent the ~20% decrease in the number of TECs (CD45⁺ EpCAM⁺MHC class II [MHC II⁺]) observed in BMT mice. As shown in Fig. 4A, treatment with optimal (15 μg) doses of rIL-7/HGFβ proportionately increased the numbers of total, cortical (CD45⁺ EpCAM⁺MHC II⁺Ly5¹), and medullary (CD45⁺ EpCAM⁺MHC II⁺Ly5¹) TECs by ∼50% above normal levels. Suboptimal (6 μg) doses of rIL-7/HGFβ increased the numbers of TECs to normal levels (Supplemental Fig. 3). However, neither optimal (5 μg) nor suboptimal (2 μg) doses of rIL-7 increased the number of TECs significantly (p > 0.05). As with thymocytes (Fig. 2B), rHGFβ alone or mixed with rIL-7 had no detectable effects on TEC development. Yet, Ab to c-Met inhibited the stimulatory effects of rIL-7/HGFβ on TECs by 50–70% (Fig. 4B), again suggesting that HGFβ becomes functional when incorporated into the rIL-7/HGFβ hybrid cytokine. In contrast, anti–IL-7Rα Ab did not inhibit the stimulation of TECs (Fig. 4B), which do not express IL-7Rα (Fig. 1L).

rIL-7/HGFβ efficiently increases the numbers of naive donor-origin T cells in the periphery of BMT mice

Ideally, cytokine-enhanced thymocytopenies should result in the release of naive T cells to the periphery following BMT. We therefore compared the effects of rIL-7 and rIL-7/HGFβ treatment on T cell repopulation in the spleen 30 d after BMT. As shown in Fig. 5A–D, optimal doses of rIL-7/HGFβ (15 μg) increased the numbers of total donor-origin CD4⁺ and CD8⁺ T cells by 4–7-fold above those in PBS-treated BMT mice, whereas optimal doses of rIL-7 (5 μg) increased the numbers by 2- to 3-fold. In each instance, 70–80% of the donor-origin T cells had a CD62L⁺CD44⁺ phenotype, characteristic of naive T cells newly derived from the thymus (37, 38). Hence, rIL-7/HGFβ fully restored the numbers of naive donor-origin T cells to normal, whereas rIL-7 only partially restored their numbers (~50%).

In addition to stimulating the increased generation of naive donor-origin T cells after BMT, rIL-7/HGFβ also significantly increased the number of host-origin T cells, few of which had a naive phenotype (Fig. 5E, 5F). These results suggested that rIL-7/HGFβ, like rIL-7 (14–20), was able to induce the homeostatic expansion of peripheral T cells. Moreover, as the number of total donor-origin T cells exceeded that of naive donor-origin T cells by about one third (Fig. 5A–D), the results suggested that rIL-7/HGFβ could induce naive T cells to assume a more mature phenotype.

When a suboptimal dose of rIL-7/HGFβ (6 μg) was used, the number of naive donor-origin T cells was increased by 2-fold,
Discussion

The present results demonstrate that rIL-7/HGFβ efficiently restores the pool of naive peripheral T cells in lethally irradiated mice after BMT both by inducing enhanced thymopoiesis and stimulating homeostatic expansion of recent thymic emigrants (RTEs). Importantly, the diversity of the resulting TCR repertoire of the donor-origin T cells was indistinguishable from that observed for T cells from normal control mice, suggesting that their precursors in the thymus had undergone essentially normal positive and negative selection.

Although rIL-7 also enhances T cell regeneration after BMT, there is considerable controversy as to whether this is a thymus-dependent or thymus-independent phenomenon. Several studies have concluded that rIL-7 enhances T cell regeneration primarily by stimulating thymopoiesis after BMT (10–13), whereas other studies have found that rIL-7 enhances T cell regeneration by directly stimulating the proliferation, differentiation, and survival of peripheral T cells (including RTEs) (14–19, 39, 41, 42). Similar results have been obtained in nonhuman primates and human patients (15, 17, 19).

The present experiments suggest that these discrepancies most likely are due to differences in the doses of rIL-7 administered, suboptimal doses primarily having extrathymic effects and optimal doses having both intrathymic and extrathymic effects. This explanation is consistent with the observed dose-dependent effects of IL-7 on thymocyte development in IL-7 transgenic mice (43). However, a less obvious mechanism exists by which high doses of rIL-7 might affect thymopoiesis, namely the formation of functional rIL-7/HGFβ complexes of exogenous rIL-7 with endogenous HGFβ in the presence of heparan sulfate-like molecules in the extracellular matrix (7).

The precise mechanisms by which rIL-7/HGFβ enhances thymopoiesis after BMT remain to be determined. We have previously demonstrated that rIL-7/HGFβ selectively stimulates the proliferation of early B-lineage cells by cross-linking IL-7Rα and c-Met on the cell surface, thereby triggering downstream signal cross talk and novel functional readouts (8). Hence, as rIL-7/HGFβ also stimulates thymocyte proliferation in vitro (8), and as it cross-links IL-7R and c-Met on dual receptor-expressing thymocytes, we were not surprised by its stimulatory effects on DN and SP thymocytes. However, the mechanisms by which rIL-7/HGFβ stimulates ETPs, DP thymocytes, and TECs are less clear, as none of these cell types expresses the IL-7Rα chain or responds directly to rIL-7 (32, 33). Furthermore, unlike rIL-7/HGFβ, neither rHGFβ (the current study) nor rHGF (21) detectably enhanced thymopoiesis after syngeneic or autologous BMT. Thus, when compared with its component cytokines, the effects of rIL-7/HGFβ on thymopoiesis after BMT appear to be unique.

A possible explanation for the stimulatory effects of rIL-7/HGFβ on ETPs and DP thymocytes is that it acts directly on the target cell by inducing juxtacrine signaling by cross-linking c-Met and
a cytokine receptor other than IL-7Rα (44, 45). Alternatively, it is possible that rIL-7/HGFβ acts indirectly on ETPs by increasing the generation of their precursors in the BM and/or by supporting the migration of these precursors to the thymus. This possibility is supported by our observations (8) (L. Lai and I. Goldscheider, unpublished observations) that rIL-7/HGFβ markedly enhances hematopoiesis and the generation of common lymphoid progenitors and by reports that rIL-7 and HGF upregulate chemokine receptors and adhesion molecules on a variety of lymphohematopoietic cells (46–50). It can similarly be speculated that the effect of rIL-7/HGFβ on DP thymocytes is secondary to its stimulation of DN thymocytes.

Similar explanations can be offered for the ability of rIL-7/HGFβ to induce increased numbers of TECs, which also lack IL-7Rα. Again, rIL-7/HGFβ may directly induce the survival, proliferation, and/or the differentiation of TECs or their progenitors (21). The possibility that TECs are stimulated indirectly by the activation of thymocytes is less likely given the inability of anti-IL-7Rα Ab to inhibit the effects of rIL-7/HGFβ.

Although optimal doses of both rIL-7 and rIL-7/HGFβ stimulated thymopoiesis after BMT, rIL-7/HGFβ was more effective at all doses at which molar equivalents were compared. This difference was qualitative as well as quantitative, as rIL-7/HGFβ, unlike rIL-7, increased the numbers of ETPs and TECs to levels exceeding those of normal WT mice and proportionately expanded the four major subsets of thymocytes at all dose levels (Table I). It is also possible that rIL-7/HGFβ is better than rIL-7 at enhancing thymocyte survival, as both HGF and IL-7 have anti-apoptotic properties (51, 52) and as HGF can upregulate the production of IL-7 by TECs (53). Another advantage may be the 7-fold greater in vivo t1/2 of rIL-7/HGFβ over rIL-7, presumably reflecting the higher m.w. of rIL-7/HGFβ. This is consistent with previous reports that rIL-7 has a short serum half-life (47, 54). It is notable that a single dose regimen of rIL-7/HGFβ induces a dramatic increase in ETPs, and a preferential expansion of naive T cell subsets.

In summary, the thymopoietic effects of rIL-7/HGFβ depend on the dose of cytokine administered. With low doses of rIL-7/HGFβ, we observed a preferential expansion of pro- and pre-TCRαβ+ DP thymocytes at all dose levels. With high doses of rIL-7/HGFβ, we observed a preferential expansion of pro- and pre-TCRδ+ DP thymocytes at all dose levels. This difference in thymopoietic effects may be due to differences in the affinity of the IL-7Rα/ββ and IL-7Rα/βγ receptors for rIL-7/HGFβ.


