LETTER TO THE EDITOR

LMO2 and IL2RG synergize in thymocytes to mimic the evolution of SCID-X1 gene therapy-associated T-cell leukaemia

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The SCID-X1 disease occurs in males that lack a functional X-linked gene encoding the interleukin 2 receptor subunit gamma (IL2RG) and thus are immuno-deficient (reviewed in Rochman et al.). Gene therapy has been a success in curing SCID-X1 in patients receiving autologous CD34+bone marrow cells infected with retroviruses expressing IL2RG. This treatment protocol has, however, produced adverse T-cell effects where clonal T-cell leukaemias arose, and four have insertional mutagenesis of the T-cell oncogene LMO2.1–3 LMO2 is a T-cell oncogene first discovered via chromosomal translocations in T-cell acute leukaemia (T-ALL) (reviewed in Chambers and Rabbitts). It is unclear if the T-cell neoplasias in the SCID-X1 patients are simply due to insertional activation of the LMO2 gene or reflect synergy between LMO2 and IL2RG. Further, the recurrent involvement of LMO2 in SCID-X1 leukaemias is puzzling as other T-cell oncogenes (for example, TAL1/SCL, HOX11 and LYL1) might equally have been targets. This suggests that specific properties of LMO2 per se are required in these adverse events. The oncogenic potential of IL2RG itself also remains controversial. Although it causes T-cell lymphomas in mice transplanted with virally transduced haematopoetic stem cells, other studies have indicated that IL2RG is not an oncogene.1 Here we provide evidence that synergy is required between LMO2 and IL2RG proteins specifically in the T-cell lineage to elicit neoplasias and that additional mutations are required such as Notch1 mutations like those in human T-ALL.12

We made two new transgenic strains where Lmo2 or IL2RG expression uses the Lck-promoter vector (summarised in Figure 1a) to express in thymus T cells. Lck-Lmo2 transgene expression was confirmed using deep sequencing RNA-seq (Ruggero and Rabbitts, manuscript in preparation), and expression of the IL2RG transgene was confirmed by flow cytometry (Supplementary Figure 1a). Lck-Lmo2 expression causes a T-cell differentiation blockage at the CD4; CD8 double-negative stage (DN cells) (Supplementary Figure 1d) as first shown with CD2-Lmo2 mice.13,14 Expression of the Lck-IL2RG transgene alone did not affect the phenotype of the thymus population (Supplementary Figure 1c).

Double transgenic mice expressing both LMO2 and IL2RG were generated by interbreeding. Both single Lck-Lmo2 and double Lck-Lmo2; Lck-IL2RG transgenic lines develop clonal T-cell neoplasias characterised by thymoma and splenomegaly, after a latency period of several months. Kaplan–Meier survival curves are shown in Figure 1b. We note a significant difference in the time of appearance of T-cell thymomas in the Lck-Lmo2 mice (mean occurrence of 320 days) compared with the double transgenic Lck-Lmo2; Lck-IL2RG counterparts (mean occurrence of 220 days). The Lck-IL2RG single transgenic mice did not show signs of ill health or alteration of thymocyte differentiation. Histological analysis of the single and double transgenic mouse tumours showed similar pathology with homogeneous cellularity in the thymus, loss of distinct red and white pulp in the spleen, and perivascular deposits in pale liver and kidneys (Supplementary Figures S2 and S3). Thus, thymic co-expression of LMO2 and IL2RG proteins synergistically accelerates the rate of T-cell tumour formation compared with LMO2 alone.

T-cell tumours in Lck-Lmo2 and Lck-Lmo2; Lck-IL2RG mice were clonal as judged by Southern hybridisation analysis of T-cell receptor β-chain (Tcrb) gene rearrangements in thymoma genomic DNA (Supplementary Figures 4a and b). Most of the tumours analysed display rearrangement of one or both Tcrb alleles. The DN surface phenotype of the asymptomatic cases was essentially the same between strains, and the tumours were transplantable. A representative single Lck-Lmo2 mouse (STG21) and two double transgenic mice (DTG21 and DTG22) were compared in more detail by amplification of unique genomic polymerase chain reaction (PCR) products using Vβ and Jβ PCR primers (Supplementary Figure 4c) demonstrating bi-allelic Tcrb rearrangements. STG21 has a DN2 phenotype (Figure 1d) similar to the CD2-LMO2 strain.13–15 The two double transgenic Lck-Lmo2; Lck-IL2RG mice (DTG21 and DTG22, Figures 1e and f) had either a mixed DN1 and DN2 phenotype or a DN3 phenotype.

The long latency of tumours in the transgenic mice shows that LMO2 is necessary, but not sufficient for T-cell neoplasia and other genetic or epigenetic changes must occur for overt neoplasia. Furthermore, the decrease in the length of this asymptomatic period in Lck-Lmo2; Lck-IL2RG mice indicates the accumulation of these mutations occurs more rapidly and/or different mutations account for the rate increase. A number of mutations have been identified in LMO2-associated human T-ALL, including NOTCH1 mutations that appear in nearly half of cases.12 We analysed the Notch1 gene in the transgenic tumours and found mutation in 65 and 55% of samples, respectively, of the single and double transgenic tumours. Two types of mutation were observed (Figure 2). A hot spot for mutations was identified in exons 26–27 (Figure 2a) that code for the heterodimerisation domain, responsible for the non-covalent interaction between the trans-activator and the extracellular domains. A second set of common mutations were insertions in exon 34 coding for the proline, glutamic acid, serine, threonine-rich sequence (PEST) domain (Figure 2b). This region of the Notch1 protein regulates degradation and mutations, therein affecting the protein half-life.12 The mutations observed in our mouse T-cell neoplasias (both the Lck-Lmo2 and Lck-Lmo2; Lck-IL2RG lines) faithfully recapitulate the mutations observed in human T-ALL.12 Thus, transit from asymptomatic and differentiation-blocked thymocytes to clonal neoplasia results from Notch1 gene mutation in at least half of the cases.12

In establishing the dual transgenic model of LMO2 and IL2RG, we sought to elucidate the roles of these two genes in the adverse effects encountered in patients receiving SCID-X1 gene therapy. Our data show that IL2RG is not directly oncogenic in T cells. However, LMO2 and IL2RG functionally co-operate in thymocytes
to accelerate tumourigenesis as IL2RG shortens the period in which cells acquire mutations required to drive leukaemia. Most likely, because LMO2 invokes thymocytes differentiation block at DN2/DN3 stages where IL2RA (CD25) is expressed, linking its effect to the other IL2R chain, IL2RG. It is intriguing as IL2RG is required to form a higher affinity IL2 receptor with IL2RA, whose downstream signalling affects T-cell proliferation and differentiation.1

The LMO2 and IL2RG co-operation that causes the faster tumour aetiology may be a consequence of LMO2-induced blockade at DN2/3 and expression of transgenic IL2RG resulting in IL2 receptor precluding these cells entering a prolonged resting state. Finally, it is noteworthy that not all SCID-X1 gene therapy patients developed leukaemia, although some had clones with detectable retroviral insertion at LMO2.16 This is presumably because LMO2 is not sufficient for T-cell oncogenesis14 and secondary mutations are needed for overt disease, such as Notch1. In our transgenic model, the high penetrance of T-cell neoplasia reflects the high number of T cells available for random secondary mutations to occur.

In our model, ectopic expression of LMO2 and IL2RG is restricted to the T-cell lineage, establishing that co-expression of these genes in SCID-X1 therapy patients is sufficient to drive development of leukaemia. Although SCID-X1 patients with the requisite retroviral insertion may co-express LMO2 and IL2RG in bone marrow pluripotent cells, it seems likely that leukaemogenesis is only initiated once those cells arrive in the thymus to be subjected to the LMO2-mediated differentiation block. Thus, LMO2 gene expression in bone marrow progenitors is not relevant per se, but rather the probability of inserting into any open chromatin gene is related to the dose of transducing retrovirus and the number of CD34+ HSC transduced for the gene therapy.7 In Wiskott–Aldrich gene therapy, patients also developed leukaemias showing LMO2 activation through retroviral insertion.17 Interestingly, several of these leukaemias had secondary insertions at the TAL1 gene, known to enhance tumourigenesis in mice,14 or the LYL1 gene encoding another LMO2 interaction partner.18

Correction of the IL2RG deficiency in SCID-X1 is a significant success that was tempered when patients developed therapy-
related T-cell leukaemia. It is now clear that the adverse T-cell effects in the patients resulted from an unexpected consequence of insertional mutagenesis into a gene (LMO2) that has the power, when aberrantly activated, to affect the differentiation of the very cells that are defective in SCID-X1.

**CONFLICT OF INTEREST**

The authors declare no conflict of interest.

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Supplementary Information accompanies this paper on the Leukemia website (http://www.nature.com/leu)