The current findings in the mouse appear relevant for human T-ALL. The (partial) loss of Tcf1 is now also reported in a subset of pediatric T-ALL, the ETP group, in which deletions of *TCF7* were found in two patients (Yu et al., 2012). *MEF2C* has recently been shown to act as the master transcriptional regulator of genes that constitute the signature of ETP-ALL (Homminga et al., 2011). Interestingly, gene expression profiling of *Tcf7*$^{-/-}$ lymphomas shows upregulated expression of *Mef2c*(Tiemessen et al., 2012), further supporting the possibility that lack of Tcf1 may be involved in development of certain ETP-ALL cases.

The unexpected finding that Tcf1 is a tumor suppressor gene also indicates that Tcf1 and Lef1 are not merely redundant in the thymus. For normal T cell development, a deficiency in both Lef1 and Tcf1 results in a more profound arrest in thymocyte differentiation than seen with Tcf1 alone (Yu et al., 2012), indicating a redundant role of these two factors. However, for the development of T cell lymphomas, Lef1 is the culprit leading to cellular transformation, whereas Tcf1 can function as transcriptional repressor of Lef1, in the role of a classical tumor suppressor gene. How this dichotomous cooperative although antagonistic relationship between these two DNA-binding factors is regulated and if Wnt signaling plays a role in this regulation is currently not understood (Figure 1, bottom).

Tcf1 has been shown to act as commitment factor for the T cell lineage in hematopoietic stem cells in gain-of-function experiments (Weber et al., 2011), a finding that was not corroborated by the loss-of-function experiments reported by Yu et al. (2012). This may indicate that yet another Tcf related factor, for instance Tcf4, can partially compensate for loss of both Tcf1 and Lef1, because inhibition of canonical Wnt signaling blocks thymopoiesis at DN1. Whether the dual roles that Tcf1 plays in T cell development are intrinsically different from the roles of Lef1 or merely determined by stoichiometry and abundance of various splice variants (in different lineages or developmental stages) remains to be determined. Nevertheless, the T cell-specific tumor suppressor function of Tcf1 that is best known as an activator of the Wnt pathway is an unexpected twist.

REFERENCES

Clevers, H. (2006). Cell *127*, 469–480.

Guo, Z., Dose, M., Kovalovsky, D., Chang, R., O'Neil, J., Look, A.T., von Boehmer, H., Khazaie, K., and Gounari, F. (2007). Blood *109*, 5463–5472.

Homminga, I., Pieters, R., Langerak, A.W., de Rooi, J.J., Stubbs, A., Verstegen, M., Vuerhard, M., Buijs-Gladdines, J., Kooi, C., Klous, P., et al. (2011). Cancer Cell *19*, 484–497.

Luis, T.C., Ichii, M., Brugman, M.H., Kincade, P., and Staal, F.J. (2012). Leukemia *26*, 414–421.

Luis, T.C., Naber, B.A., Roozen, P.P., Brugman, M.H., de Haas, E.F., Ghazvini, M., Fibbe, W.E., van Dongen, J.J., Fodde, R., and Staal, F.J. (2011). Cell Stem Cell *9*, 345–356.

Roose, J., Huls, G., van Beest, M., Moerer, P., van der Horn, K., Goldschmeding, R., Logtenberg, T., and Clevers, H. (1999). Science *285*, 1923–1926.

Staal, F.J., Luis, T.C., and Tiemessen, M.M. (2008). Nat. Rev. Immunol. *8*, 581–593.

Tiemessen, M.M., Baert, M.R., Schonewille, T., Brugman, M.H., Famili, F., Salvatori, D., Meijerink, J.P., Ozbek, U., Clevers, H., Dongen van, J.J.M., and Staal, F.J.T. (2012). PLoS Biol. Published online November 27, 2012. http://dx.doi.org/10.1371/journal.pbio.1001430.

Weber, B.N., Chi, A.W., Chavez, A., Yashiro-Ohtani, Y., Yang, Q., Shestova, O., and Bhandoola, A. (2011). Nature *476*, 63–68.

Yu, S., Zhou, X., Steinke, F.C., Liu, C., Chen, S.C., Zagorodna, O., Jing, X., Yokota, Y., Meyerholz, D.K., Mullighan, C.G., et al. (2012). Immunity *37*, this issue, 813–826.

Retroviral Danger from Within: TLR7 Is in Control

Arun K. Mankan[1] **and Veit Hornung**[1,*]
[1]Institute for Clinical Chemistry and Clinical Pharmacology, Unit for Clinical Biochemistry, University Hospital, University of Bonn, 53127 Bonn, Germany
*Correspondence: veit.hornung@uni-bonn.de
http://dx.doi.org/10.1016/j.immuni.2012.10.009

In this issue of *Immunity*, Yu et al. (2012) outline a fascinating model in which TLR7-mediated antibody production acts as a dominant immunosurveillance mechanism against endogenous retroviruses (ERVs), with additional support of TLR3 and TLR9 that function to prevent ERV-mediated malignancy.

Our innate immune system has evolved several pattern recognition receptors (PRRs) to sense microbial nucleic acids. A prominent group of these PRRs constitutes the subfamily of Toll-like receptors (TLRs) that has evolved to sense ssRNA and short dsRNA (TLR7, TLR8), DNA (TLR9), or long dsRNA (TLR3). Upon ligand recognition, TLR activation sets off signaling cascades that are geared toward the eradication of the microbial pathogen, ranging from the activation of immediate effector functions to the initiation of adaptive immune responses (Kawai and Akira, 2011).

A large proportion of the mammalian genome is made up of fossils of

transposable elements and a major part of these remnants are derived from endogenous retroviruses (ERVs) (Stocking and Kozak, 2008; Stoye, 2012). ERVs are the result of exogenous retroviruses (XRVs) that have integrated within the germline of the host, thereby subjecting the provirus to mendelian inheritance. Most ERVs have lost their capability to propagate because of numerous inactivating mutations, yet a considerable number of active ERVs has been documented in the murine system (Stoye, 2012). Among these, the best-studied ERVs can be found within the class I ERVs that are closely related to the gamma retrovirus genus of XRVs, typified by murine leukemia viruses (MuLVs). MuLVs are well known for their ability to induce oncogenic transformation leading to leukemia in mice and as such they have been studied extensively. Germline-encoded provirus, with the potential of giving rise to infectious MuLVs with ecotropic host range, are found in low copy number in various inbred mouse strains, and the commonly used laboratory strain C57BL/6 harbors the provirus of a defective endogenous ecotropic MuLV (E-MuLV) on chromosome 8 (*Emv2*). *Emv2* harbors a point mutation in its *pol* region that prevents its propagation a priori. In addition, its so-called N-tropism encoded in its *gag* region would restrict its growth in C57BL/6 mice that express the allele combination Fv-$1^{b/b}$, which encodes for a restriction factor for N-tropic viruses (Stocking and Kozak, 2008). Mechanistically, *Emv2* reactivation is most probably due to in *trans* complementation of its defects by nonecotropic ERVs, an event that can be triggered by genetic (crossbreeding) or environmental (e.g., inflammation) factors (Greenberger et al., 1975; McCubrey and Risser, 1982). Nonetheless, under normal conditions inbred C57BL/6 mice do not display E-MuLV viremia that would lead to disease pathology. Next to dedicated restriction factors that prevent propagation of endogenous and exogenous MuLVs in a cell-intrinsic fashion (Stoye, 2012), it has been shown that exogenous MuLV infection in ERV-negative inbred mouse strains is effectively controlled by a TLR7-dependent antibody response (Browne, 2011; Kane et al., 2011).

In order to dissect the exact role of the three nucleic acid-sensing TLRs, Yu et al. (2012) generated mice deficient in these TLRs. Unexpectedly, they found that aged *Tlr3*$^{-/-}$*Tlr7*$^{-/-}$*Tlr9*$^{-/-}$ mice were overtly sick and died earlier when compared to the age-matched wild-type controls. Autopsy of diseased animals showed enlarged thymus and secondary lymphoid organs and histology revealed a pre-T cell acute lymphoblastic lymphoma (T-ALL). Microarray analysis of splenic tissue in *Tlr3*$^{-/-}$*Tlr7*$^{-/-}$*Tlr9*$^{-/-}$ mice showed a dramatic upregulation of endogenous retroviral sequences, the presence of which was confirmed by the visualization of the budding viruses from the thymic tissue. To identify the contribution of the individual endosomal TLRs to this phenotype, Yu et al. (2012) checked for active ERVs in different single and compound mutant mice. *Tlr7*$^{-/-}$ and compound mutant mice where TLR7 was also deleted showed significant increase in ERV mRNA and protein expression. However, no evidence of ERV viremia was observed in *Tlr3*$^{-/-}$, *Tlr9*$^{-/-}$, or *Tlr3*$^{-/-}$*Tlr9*$^{-/-}$ mice, indicating that absence of TLR7 alone was sufficient and necessary for the virus to propagate. In line with this notion, mice deficient in upstream or downstream components of the TLR7 signaling cascade also displayed increased retroviral gene expression. However, whereas TLR7 deficiency led to retroviremia in these mice, only *Tlr3*$^{-/-}$*Tlr7*$^{-/-}$*Tlr9*$^{-/-}$ animals displayed T-ALLs. Subsequent studies indicated that none of the T cell populations within the thymus showed significant endosomal TLR expression, yet the cortical thymic epithelial cells and plasmacytoid dendritic cells expressed them, suggesting that TLR-mediated protection against ERV propagation operates in a cell-extrinsic manner.

The pol region within retroviral RNAs from T-ALL lines revealed strong homology to the endogenous *Emv2* provirus, with a correction of its mutation. This is highly indicative of recombination events between *Emv2* and one or more nonecotropic viruses subsequently giving rise to a replication competent retrovirus. Studying ERV integration at the genomic level, the authors observed that *Tlr3*$^{-/-}$*Tlr7*$^{-/-}$*Tlr9*$^{-/-}$ mice displayed multiple integrations events in lymphoid tissue and most prominently in tumor cell clones. Surprisingly, yet in line with the tumor data, only *Tlr3*$^{-/-}$*Tlr7*$^{-/-}$*Tlr9*$^{-/-}$ mice, but no other ERV viremic mice (e.g., *Tlr7*$^{-/-}$), showed additional proviral integrations at the genomic level, at least when using Southern blot to probe for such events. A common driver in retrovirus-mediated oncogenesis is the activation of proto-oncogenes by the integration of proviral DNA in vicinity to these genes. In line with this, the authors observed frequent genomic ERV reintegration events within proximity to coding sequences of proto-oncogenes in T-ALL cell lines of *Tlr3*$^{-/-}$*Tlr7*$^{-/-}$*Tlr9*$^{-/-}$ mice.

Analyzing the serum from different genotypes for the presence of anti-ERV-specific IgG antibodies revealed an inverse picture as compared to the ERV expression data. Mice devoid of active ERVs displayed high amounts of anti-ERV-specific IgG antibodies, and these antibodies were also able to inhibit the replication of an exogenous MuLV in vitro. Interestingly, despite comparable titers, anti-ERV serum from wild-type mice showed the highest inhibitory activity, whereas serum from *Tlr3*$^{-/-}$, *Tlr9*$^{-/-}$, or *Tlr3*$^{-/-}$*Tlr9*$^{-/-}$ mice was less effective.

Yu et al. (2012) suggest that de novo ERV reactivation occurs spontaneously in wild-type C57BL/6 mice (Figure 1). The exact location, timing, and mechanism of this reactivation is unclear, yet its occurrence is unequivocally documented by the high and specific class-switched ERV antibody titers. This is in line with early studies that have reported the phenomenon of ''autogenous'' anti-ERV immunity in inbred mouse strains in the absence of retroviremia (Hanna et al., 1972). The fact that TLR7 plays a nonredundant role in the induction of protective antibody titers in response to ERV reactivation is intriguing, yet not unexpected given previous studies exploring the role of TLR7 in adaptive immune responses toward XRVs (Browne, 2011; Kane et al., 2011). However, Yu et al. (2012) refine and extend this concept by demonstrating a nonredundant role for TLR7 in the immunosurveillance of endogenous retroviruses. Extrapolating results from studies with exogenous MuLVs predicts that B cell-intrinsic TLR7 activation is instrumental for this protection (Browne, 2011).

Theoretically, loss of TLR7 signaling could also be positioned upstream of the observed ERV reactivation event through

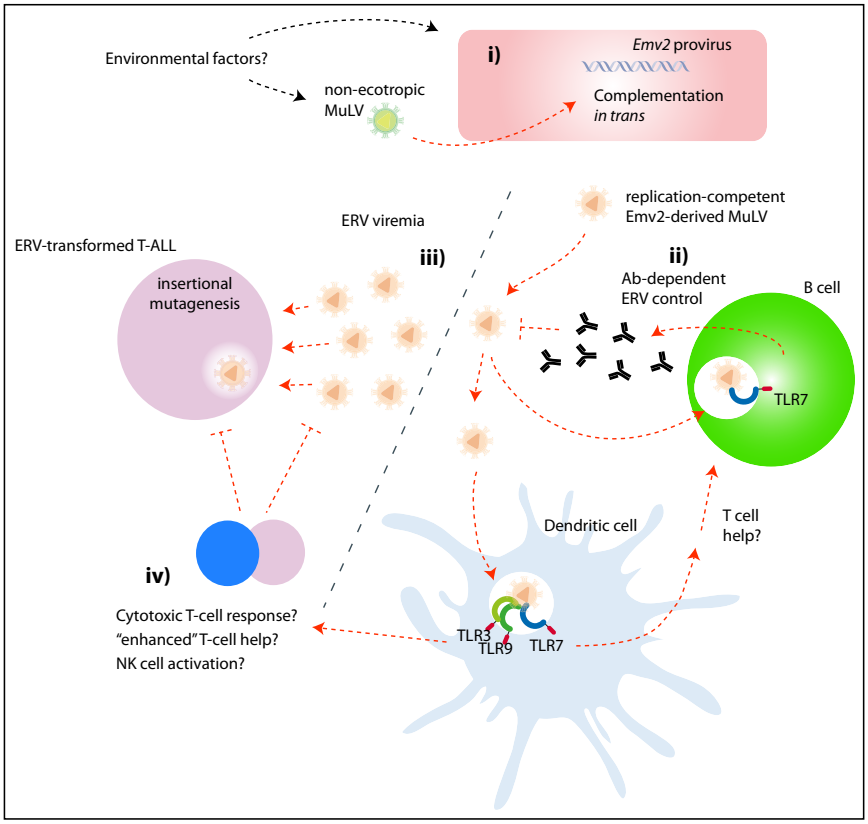

Figure 1. A Schematic View of the Role for TLR3, TLR7, TLR9 in Controlling Endogenous Retroviruses

(i) Through yet unknown mechanisms, the C57BL/6 ERV provirus *Emv2* is reactivated, most probably resulting from a complementation event in *trans* by nonecotropic ERVs. Environmental factors could play an important modulatory role in this process. (ii) ERVs activate TLR7 in B cells and/or dendritic cells, leading to an adaptive immune response that results in the production of high ERV antibody titers that function to neutralize and control ERVs. (iii) The absence of TLR7 or TLR7 signaling results in the loss of protective ERV antibodies and subsequently in retroviremia, yet this is kept in check by TLR3- and TLR9-dependent mechanisms. (iv) Possible effector mechanisms that are governed by TLR3 and TLR9 could be activation of cytotoxic $CD8^+$ T cells, or $CD4^+$ T cells and/or NK cells that eradicate retrovirally infected cells.

an indirect mechanism that is unrelated to the role of TLR7 in orchestrating the antiretroviral antibody response. However, this scenario appears less likely given the well-documented role of TLR7 in directing antiretroviral immunity and the strict inverse correlation of ERV-specific antibody titers and viremia as a function of TLR7 expression. Nevertheless, it has to be considered that a dysfunctional TLR7 axis and more so a complete lack in nucleic acid-sensing TLRs could also have an impact on the reactivation and propagation of the ERVs themselves, independently of the specific anti-ERV response. Indeed, it is conceivable that *Tlr3*$^{-/-}$*Tlr7*$^{-/-}$*Tlr9*$^{-/-}$ animals display a dysfunctional immune system that could lead to secondary events facilitating the reactivation of ERVs. Indeed, in a recent study by Young et al. (2012), the lack of functional B cell immunity was also identified as a critical component for ERV reactivation. However, Young et al. (2012) positioned lack of antibody production upstream of ERV reactivation rather than functioning as an endogenous ERV surveillance mechanism. More specifically, a dysregulation of the intestinal microbiome in the absence of B cell immunity was suggested to trigger inflammation leading to ERV reactivation. Further studies will be required to reconcile these two models.

Another intriguing finding is that only the additional absence of TLR3 and TLR9 results in tumorigenesis in ERV-viremic, TLR7-deficient animals. The authors suggest that this could be due to a qualitative difference in the adaptive immune response of the host. Indeed, neutralization experiments with sera from wild-type versus *Tlr3*$^{-/-}$, *Tlr9*$^{-/-}$, or *Tlr3*$^{-/-}$*Tlr9*$^{-/-}$ mice alludes to a qualitative difference of the anti-ERV sera. Moreover, the different repertoire of antigens being recognized by these sera points into the same direction. As such, it is possible that T cell help provided indirectly by TLR3 and TLR9 activation drives the formation of a more effective antibody repertoire. Another likely scenario is that TLR3 and TLR9 could be instrumental for the initiation of cytotoxic $CD8^+$ T cell responses, a phenomenon that is known to be independent of TLR7 in the context of exogenous MuLV infection (Browne, 2011). TLR3, for example, plays an important role in cross priming antigen-specific $CD8^+$ T cells and it is possible that this mechanism accounts for the induction of cytotoxic T cell responses eliminating transformed T cells. At the same time, it should also be considered that the additional absence of TLR3 and/or TLR9 could indirectly shape a different MuLV "repertoire," which could lead to the observed malignant transformation. In this respect, it would be very informative to obtain full sequences of the ERV repertoire arising in the different gene-deleted mouse strains.

In summary, Yu et al. (2012) unravel a pivotal role for nucleic acid-sensing TLRs in the immunosurveillance of endogenous retroviruses, with TLR7 playing a dominant role in inhibiting ERV viremia in the first place and TLR3 and TLR9 playing a secondary but significant role in preventing retroviral-induced malignancy. In the absence of clear evidence showing a role for ERVs in human diseases, it remains speculative whether similar protective mechanisms are at play in the human system and how they impact disease. Nevertheless, primed by this study we may now be looking for a possible protective role for ERV immunosurveillance in humans.

REFERENCES

Browne, E.P. (2011). PLoS Pathog. *7*, e1002293.

Greenberger, J.S., Phillips, S.M., Stephenson, J.R., and Aaronson, S.A. (1975). J. Immunol. *115*, 317–320.

Hanna, M.G., Jr., Tennant, R.W., Yuhas, J.M., Clapp, N.K., Batzing, B.L., and Snodgrass, M.J. (1972). Cancer Res. *32*, 2226–2234.

Kane, M., Case, L.K., Wang, C., Yurkovetskiy, L., Dikiy, S., and Golovkina, T.V. (2011). Immunity *35*, 135–145.

Kawai, T., and Akira, S. (2011). Immunity *34*, 637–650.

McCubrey, J., and Risser, R. (1982). J. Exp. Med. *156*, 337–349.

Stocking, C., and Kozak, C.A. (2008). Cell. Mol. Life Sci. *65*, 3383–3398.

Stoye, J.P. (2012). Nat. Rev. Microbiol. *10*, 395–406.

Young, G.R., Eksmond, U., Salcedo, R., Alexopoulou, L., Stoye, J.P., and Kassiotis, G. (2012). Nature.

Yu, P., Lübben, W., Slomka, H., Gebler, J., Konert, M., Cai, C., Neubrandt, L., Prazeres da Costa, O., Paul, S., Dehnert, S., et al. (2012). Immunity *37*, this issue, 867–879.

Langerhans Cells Come in Waves

Nikolaus Romani,[1,*] Christoph H. Tripp,[1] and Patrizia Stoitzner[1]
[1]Department of Dermatology and Venereology, Innsbruck Medical University, Anichstrasse 35, A-6020 Innsbruck, Austria
*Correspondence: nikolaus.romani@i-med.ac.at
http://dx.doi.org/10.1016/j.immuni.2012.10.013

It is unclear how the Langerhans cell (LC) network is maintained in adult epidermis. In this issue of *Immunity*, Seré et al. (2012) show that LCs are replenished in two waves. Monocyte-derived, short-lived LCs come first. A second wave follows, and these LCs of nonmonocytic origin are long-lived.

Langerhans cells (LCs) are dendritic cells of epithelia. The best-studied example is LCs of the epidermis (Romani et al., 2010). From the early experiments, it has been appreciated that there is something special about the life cycle of epidermal LCs. When human skin was transplanted onto nude mice, human LCs within the graft persisted for a remarkably long period of several months (Krueger et al., 1983). Studies from others have corroborated these observations (Merad et al., 2002). It was established that (1) LCs populate the epidermis early in ontogeny and (2) they are very long-lived. The LC network is established within a few days after birth by an initial burst of proliferation as demonstrated in murine epidermis (Chorro et al., 2009). In human skin, precursors of LC are present in the embryonic skin, and they gradually acquire LC-specific markers during development of the fetus (Schuster et al., 2009). With regard to longevity, LCs essentially persist for the entire lifetime of a mouse, provided the epidermis is not disturbed by inflammation. Even in human hand transplants, the LCs of the hand donor remained in place for almost a decade (Kanitakis et al., 2011).

From this body of evidence, one could infer that LCs in the steady-state adult epidermis are a largely independent cell population that renews itself and does not need fresh supplies from progenitor cells in bone marrow or the dermis. In contrast, another nonembryonic LC precursor cell has been reported (Ginhoux et al., 2006). During skin inflammation induced by UV-C irradiation, which largely depletes resident LCs from the epidermis, LCs are subsequently replenished by immigrating monocytes that are characterized by the expression of the Gr-1 marker. Once in the epidermis, monocytes acquire langerin (also known as CD207) expression.

In spite of the seminal findings mentioned above, the homeostasis of LCs in the adult epidermis is still not entirely clear. Can monocytic LC precursors fully make up for the inflammation-induced lack of LCs? Do these "monocyte-derived LCs" in the epidermis live equally long (i.e., virtually life-long) as the "normal" LCs that populated the epidermis around birth? How exactly is the epidermis reconstituted with LCs after an inflammation-induced efflux of LCs? In this issue of *Immunity*, Seré et al. (2012) made use of mice with deleted transcription factor Id2, which strikingly lack all LCs (Hacker et al., 2003) and langerin$^+$ dermal dendritic cells. Thus, the mice are devoid of any langerin-expressing cells in the skin. When wild-type (WT) bone marrow was injected into these adult Id2-deficient mice, a network of LCs established over time, suggesting that the repopulation in the steady-state occurred by bone marrow-derived precursors that acquire MHC-II first, followed by langerin expression. This is similar in the newborn. Shortly after birth and settlement in the epidermis, both markers sequentially come up on LCs (reviewed in Romani et al., 2010). The time course was protracted in the chimeric mice (few weeks after bone marrow transfer) as compared with newborn (few days after birth). The authors then undertook a set of experiments to dissect repopulation during skin inflammation, and to this end, they resorted to the UV model originally described by Merad et al., (2002). UV-C irradiation led to inflammation in the skin and to the loss of LCs from the epidermis within 1 week. At the same time, both epidermis and dermis were strongly infiltrated by Gr-1$^+$ monocytic cells. Within the following 4 weeks, the epidermis of WT mice was repopulated by dendritic cells, which acquired MHC-II and high langerin expression in a sequential fashion, as it may have seemed at first glance. Id2-deficient mice,