Commentary

TRANCE–RANK, a New Signal Pathway Involved in Lymphocyte Development and T Cell Activation

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Almost a decade ago, major advancements in our understanding of the cell–cell interactions that were critically involved in the regulation of the immune response were achieved when it was established that T cell activation not only required a signal through the T cell receptor but also a second signal generated by the interaction of costimulatory molecules CD80 and CD86 on the surface of APCs and CD28 or CTLA-4 on T cells (1). Since then, identification of other molecular interactions that are important in regulating the immune response has increased dramatically, elucidating signals that enhance stable cellular interactions between APCs and T cells as well as apoptotic signals that regulate survival of either the APC or the T cell (2, 3). One molecular interaction that has received enormous attention is that between CD40 on APCs and CD154 on T cells (reviewed in 4). CD40–CD154 signals are a vital component in the generation of humoral immunity, as exemplified by murine models in which blockade of CD40–CD154 signals, either by antibodies or the creation of a null mutation in CD154, abrogates development of B cell responses to thymus-dependent antigens (5, 6). Signals through CD40 can also influence the activity of other APCs, in particular dendritic cells (DCs), leading to the upregulation of adhesion and costimulatory molecules (7, 8), increased accumulation of peptide–MHC complexes on DC surfaces, and the generation of antiapoptotic signals that increase DC survival (9, 10), thus helping to prolong contact between DCs and T cells. Although the importance of CD40–CD154 interactions in the generation of B and T cell immunity are indisputable, findings that CD40- and CD154-deficient mice can mount T cell responses to certain viral infections have suggested a novel CD40–CD154-independent pathway involved in the activation of T cells (11–13). In this issue, Bachmann et al. (14) provide compelling evidence that this CD40–CD154-independent pathway for T cell activation involves interaction between a newly described molecule, TRANCE (TNF-related activation-induced cytokine), on T cells and its cognate receptor, RANK (receptor activator of NFκB), on DCs.

TRANCE (also called receptor activator of NFκB ligand [RANKL], osteoprotegerin ligand [TRANCE/OPGL], and osteoclast differentiation factor [ODF]) was discovered almost simultaneously by two groups during attempts to clone novel genes involved in the regulation of apoptosis and function of DCs (15, 16). TRANCE is a member of the TNF ligand superfamily that includes Fas ligand, CD27, CD30, CD154, 4-1BBL, OX40L, TNF-α, lymphotoxin (LT)α/β, and TRAIL (TNF-related apoptosis-inducing ligand). Sequence analysis has shown that the extracellular domain of TRANCE shares 18–28% amino acid identity with other members of the TNF superfamily and the greatest identity with CD154 (16). Furthermore, TRANCE has been mapped to chromosome location 13q14 in humans, where the first TNF ligand family member genes have also been located. Extensive studies relating to the pattern of expression of TRANCE mRNA demonstrated that the highest levels of mRNA were detectable in lymph nodes and restricted to T cells. The ligand for TRANCE is RANK, a member of the TNFR superfamily that shares, like TRANCE with the TNF superfamily, remarkable sequence homology with other members of the TNFR superfamily, CD40 in particular; its chromosomal location has been mapped to position 18q22.1 in humans, where TNFR family members reside. Although RANK mRNA can be detected in skeletal muscle, thymus, liver, colon, small intestine, and adrenal gland, at the protein level, RANK expression is only detectable on the surfaces of mature DCs, suggesting that RANK expression is posttranscriptionally regulated. At the cell surface level, RANK expression is dependent on the activation of T cells, as surface expression of RANK is rarely detectable in the absence of cytokines (16).

In this issue, Bachmann et al. expand on these earlier findings, demonstrating that TRANCE not only shares sequence homology to CD154 but shares several of its functional properties as well. For example, treatment of mature DCs with TRANCE increases expression of inflammatory cytokines, such as IL-1 and IL-6, and secretion of IL-12, which can promote differentiation of $CD4^+$ T cells into Th1 cells (17). Furthermore, TRANCE treatment of DCs prolongs the life span of DCs by upregulating Bcl_{XL} expression, another property shared with CD154 (18). These striking functional similarities between TRANCE and CD154 led Bachmann et al. to determine whether signaling through the TRANCE–RANK pathway could substitute for CD154–CD40 signals in the generation of immunity to viral infections. To address this, they used the well defined lymphocytic choriomeningitis virus (LCMV) infection as a murine model, as $CD4^+$ T cell responses to LCMV infections have been shown to be independent of CD154 (11). Preliminary analysis demonstrated that LCMV infection upregulated

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TRANCE on both $CD4^+$ and $CD8^+$ T cells, yet TRANCE–RANK signals were not vital for the generation of humoral or cell-mediated immunity to LCMV in mice that could utilize the CD40–CD154 signaling pathway, as blockade of TRANCE signaling by injection of soluble TRANCE-R–human IgG1 Fc fusion protein (Tr–Fc) had little impact in abrogating either type of immunity to LCMV. The same could not be said when the experiments were repeated in CD40-deficient mice. In these animals, inhibition of TRANCE signaling led to an almost complete block in the generation of $CD4^+$ T cell immunity to LCMV and total abrogation of IFN-γ production, which is normally inducible following LCMV infection of CD40-deficient mice (11). These data suggest that TRANCE–RANK signals do not override CD40–CD154 signals in the regulation of the immune response; rather, TRANCE–RANK signaling offers an alternative route for the activation of T cells.

Much of our understanding of the importance of TRANCE–RANK signals has centered on analyzing the impact such signals have on the efficacy of DCs in activating T cells, and this is due in part to the evidence that RANK expression seems to be restricted to DCs. However, there is now evidence to suggest that TRANCE is also critically involved in the development of B and T cells as well as lymph node organogenesis. This is based on the interesting studies by Kong et al. (19), who recently described the phenotype of mice carrying a null mutation for osteoprotegerin ligand (TRANCE/$opgl^{-/-}$ mice). Originally generated to address the role of TRANCE/OPGL in osteoclast differentiation, they found that, in addition to the expected osteopetrosis and defects in tooth formation, TRANCE/$opgl^{-/-}$ mice had defects in their lymphoid compartments (19). The first evidence that TRANCE/OPGL is involved in the development of the immune system was based on the observation that thymi from TRANCE/$opgl^{-/-}$ mice were significantly decreased in size. This abnormality was found to be due to a selective block in the transition from the $CD44^-CD25^+$ stage of thymocyte development to the $CD44^-CD25^+$ stage, when the TCR is first expressed. Blockade in T cell development was not absolute, and thymocytes that could bypass the blockade developed into the normal ratio of $CD4^+$ and $CD8^+$ single positive cells. The thymic abnormality in TRANCE/$opgl^{-/-}$ mice was found to be intrinsic to bone marrow–derived cells, as T cell development was normal in TRANCE/$opgl^{+/-}rag1^{-/-}$ and TRANCE/$opgl^{+/+}rag1^{-/-}$ chimeric mice, whereas TRANCE/$opgl^{-/-}rag1^{-/-}$ chimeric mice showed pronounced blockade in the transition from the $CD44^-CD25^+$ to $CD44^-CD25^+$ stage.

The necessity for TRANCE signals in the development of the immune system was not restricted to the thymic compartment. Indeed, a similar role for TRANCE in the progression of B cells from the $B220^+CD43^+$ and $B220^+CD25^-$ pro-B cell stage to the $B220^+CD43^-$, $B220^+CD25^+$, and $B220^+sIgM^+$ pre-B cell precursors in the bone marrow was seen in TRANCE/$opgl^{-/-}$ mice.

In light of this evidence that TRANCE is involved in the development of T and B cells, it can be imagined that TRANCE would also be important in the development of other bone marrow–derived cells such as DCs and monocytes. In support of this idea, it has been recently shown that monocyte cell lines can differentiate into osteoclasts if cultured with colony stimulating factor-1 and TRANCE (20, 21), suggesting that macrophages, DCs, and osteoclasts may share a common committed precursor. Furthermore, as stated above, TRANCE can act as a survival signal for DCs by enhancing expression of apoptosis inhibitor Bcl_{XL} (15). However, FACS® analysis of splenic DC populations in TRANCE/$opgl^{-/-}$ mice suggested that TRANCE was not involved in DC development, as normal numbers of $CD11c^+$ DCs were detected in the spleens of TRANCE/$opgl^{-/-}$ and TRANCE/$opgl^{+/-}$ littermates. Also, there was no suggestion that TRANCE was required for the expression of costimulatory molecules, adhesion molecules, or CD40, as similar levels of these molecules were expressed on DCs from TRANCE/$opgl^{-/-}$, TRANCE/$opgl^{+/-}$, and TRANCE/$opgl^{+/+}$ mice. In addition, functional analysis of DCs from TRANCE/$opgl^{-/-}$ mice revealed that they had the same capacity as DCs from TRANCE/$opgl^{+/+}$ mice to stimulate allogeneic T cells from normal BALB/c mice at the level of both proliferation and cytokine production. The apparent noninvolvement of TRANCE in DC development was not restricted to the DCs located in the spleen, as there were also no distinct differences in the development of DCs present in the skin and thymi of TRANCE/$opgl^{-/-}$ and TRANCE/$opgl^{+/+}$ mice. Despite this inability to detect abnormalities in the DC compartment of TRANCE/$opgl^{-/-}$ mice, T cells that have managed to overcome the thymic block are defective in their capacity to mount allogeneic responses. Thus, T cells from TRANCE/$opgl^{-/-}$ mice stimulated with allogeneic BALB/c splenic DCs showed reduced production of IL-2 and IFN-γ compared with T cells from TRANCE/$opgl^{+/+}$ mice. The defect in T cell responses was also apparent following stimulation with anti-CD3 and anti-CD28 cross-linking antibodies and occurred in both the Th1 and Th2 compartments. It would seem, therefore, that although TRANCE–RANK signals are not important for the generation of a fully functional DC, the RANK–TRANCE signal from the DC to the T cell is a critical requirement for the optimum activation of T cells.

One last, intriguing finding relating to the development of the immune system found in TRANCE/$opgl^{-/-}$ mice was that such mice lacked lymph nodes yet showed normal Peyer's patches and splenic architecture. Lymph nodes are critical components in the generation of B and T cell immunity, as it is within these unique lymphoid structures that naive T cells first encounter their cognate antigens. The molecular interactions involved in the formation and organization of lymph node architecture have been documented and involve signaling through TNF and TNFR family members, including LTα, LTβ, TNF-α, and CD40. For example, mice deficient in LTβ lack Peyer's patches and peripheral lymph nodes, but, in general, still possess mesenteric lymph nodes (22); in LTα-deficient mice, however, all lymph nodes are absent (23–26). In both of these

cited cases and in the case of animals deficient in TNFR1 or TNF, there are also aberrations in splenic architecture (27, 28), a finding that is not seen in TRANCE/opgl$^{-/-}$ mice. Thus, it would seem that TRANCE is specifically involved in the generation of lymph nodes, with no apparent role to play in the formation of Peyer's patches or the spleen. The reason for the lack of lymph node development in TRANCE/opgl$^{-/-}$ mice is still unknown, though it does not seem to relate to defects in the homing of B and T cells nor to problems associated with abnormalities in bone marrow–derived cells.

In conclusion, the findings by Bachmann et al. and Kong et al. have highlighted two novel points at which TRANCE–RANK signals have a critical role to play in the immune system. The first occurs at the earliest stages of B and T cell development in the bone marrow and thymus, respectively, where TRANCE signals are necessary for progression to the pre-B and pre-T cell stages. The second occurs in the mature immune system and is restricted to interactions between DCs and T cells. This latter role for TRANCE is intriguing. Regulating the interaction between CD40 and CD154 is one of the most vital routes to controlling immune responses and preventing the inadvertent activation of autoreactive T cells. However, certain viral infections or simple inflammatory responses can utilize a CD40–CD154-independent pathway (12, 13, 29) and lead to the generation of autoaggressive responses to self tissue. If these viruses are using the TRANCE–RANK route for activation of autoreactive T cells, then the findings presented by Bachmann et al. may provide insight into the design of novel therapeutic strategies that can circumvent CD40–CD154-independent activation of autoreactive T cells, thereby protecting against deleterious immune responses.

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