MALT1 protease: equilibrating immunity versus tolerance

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MALT1 protease links signaling cascades emanating from adaptive or innate immune receptors to the canonical NF- κ B pathway. Now, Jaworski *et al* (2014) investigate the physiological role of MALT1 protease activity in mice. Besides the expected requirement of MALT1 activity for immune activation, the study unveils a novel function for MALT1 activity for the development of peripheral tolerance. Thus, MALT1 protease can act immunogenic or tolerogenic, and this interplay will be highly relevant for the clinical development of MALT1 inhibitors.

See also: M Jaworski *et al* (December 2014)

The activation of the NF- κ B family of transcription factors in immune cells represents a key event during the establishment of an immune response. Stimulation of B- or T-cell antigen receptors (BCR/TCR) or distinct innate immune receptors promotes the assembly of the CBM complex consisting of CARMA1(CARD11)-Bcl10-MALT1 or CARD9-BCL10-MALT1. Within the CBM complex, the MALT1 scaffold serves as a bridging factor to recruit the I κ B kinase (IKK) complex and to activate canonical NF- κ B signaling (reviewed in Thome, 2008). In addition, MALT1 also contains a very unique paracaspase domain whose proteolytic activity is activated upon TCR stimulation (Coornaert *et al*, 2008; Rebeaud *et al*, 2008). Pharmacological inhibition and genetic inactivation revealed that even though MALT1 proteolytic activity is dispensable for canonical NF- κ B signaling, it is required for optimal T-cell activation (Rebeaud *et al*, 2008; Düwel *et al*, 2009). MALT1 deficiency was shown to protect mice from the induction of experimental

autoimmune encephalomyelitis (EAE) in a murine multiple sclerosis model (MS) (Brüstle *et al*, 2012; Mc Guire *et al*, 2013). Further, constitutive MALT1 activity drives survival of ABC DLBCL, one of the most aggressive B-cell malignancies (Ferch *et al*, 2009; Hailfinger *et al*, 2009). Thus, MALT1 protease is a promising drug target to treat distinct lymphomas as well as autoimmune diseases.

To evaluate the contribution of MALT1 protease activity to the physiological role of MALT1, Thome and collaborators generated a mouse model expressing catalytically inactive MALT1 by replacing the active site cysteine with alanine (MALT1^{C472A/C472A}) (Jaworski *et al*, 2014). As expected, MALT1^{C472A/C472A} mice are characterized by profound defects in both the adaptive and the innate immune responses. Indeed, despite the fact that BCR and TCR-driven activation of IKK/NF- κ B and JNK signaling is not relying on MALT1 protease activity, it is necessary for the establishment of effector functions in lymphocytes, natural killer (NK) and dendritic cells (DC). Just like in MALT1 KO mice, IL-2 production and proliferation of T cells are strongly compromised. MALT1 protease is also required for the development of marginal zone and B1 B cells, and T-cell-dependent or T-cell-independent IgM and IgG production upon immunization. Consequently, MALT1 inactivation protects mice in two T-cell-dependent autoimmune models from EAE induction as well as colitis.

Besides these expected phenotypes, MALT1^{C472A/C472A} mice beyond the age of 6 weeks displayed swollen lymph nodes and an increase in the total number of B and T cells that was much more pronounced than in MALT1 deficient mice. T cells have an

activated/effector phenotype and secrete higher levels of the Th1 and Th2 cytokines IFN- γ and IL-4. MALT1^{C472A/C472A} mice develop spontaneous autoimmune gastritis, characterized by loss of animal weight and increased serum levels of IgE and IgG1. Thome and collaborators suggest that the autoimmune phenotype is caused by impaired peripheral tolerance (Fig 1). MALT1^{C472A/C472A} mice show a severe cell intrinsic defect in the development of FoxP3⁺ regulatory T cells (Tregs). Further, adoptive transfer of functional Tregs reduced T-cell activation and ameliorated the symptoms of autoimmune gastritis in MALT1^{C472A/C472A} mice. These findings support a model in which the autoimmune phenotype in MALT1 protease defective mice is caused by residual immune activation that relies on the preserved MALT1 scaffolding function and the concomitant severe defect in peripheral tolerance as a result of reduced Treg numbers (Fig 1). In contrast, MALT1 KO mice that are characterized by even a more severe decrease in the number of Tregs are protected from the development of autoimmune gastritis, because they also completely lack immune activation due to the loss of both scaffold and protease functions (Fig 1). Hence, the autoimmune phenotype is caused by a partial TCR signaling defect in MALT1^{C472A/C472A} mice that shifts the balance from tolerance to autoimmune activation.

The reported duality of the MALT1 protease to promote cell intrinsic T-cell effector functions and to suppress activated T cells in a Treg-dependent manner raises a number of interesting questions. Even though the adoptive transfer demonstrates the potential of Tregs to counteract the autoimmunity, it remains open, if the reduced number of

Figure 1. Model for the function of MALT1 protease activity in balancing immune cell activation.

Naïve T cells activated during an immune reaction differentiate toward effector T cells (Teffs) and suppressor regulatory T cells (Tregs), and both participate in a healthy immune reaction. MALT1 scaffold and protease activity maintains an equilibrium between Teffs and Tregs (wild-type mice). Upon destruction of MALT1 scaffold and protease functions in MALT1^{-/-} mice, T-cell activation and generation of functional effector T cells as well as regulatory T cells is severely impaired, leading to immune suppression. In MALT1^{C472A/C472A} mice which are defective only in the protease function of MALT1, the residual activation of naïve T cells and the Teffs is no longer counterbalanced due to the strong accompanying reduction in Tregs. The loss of equilibrium in the T-cell compartments promotes the expansion of activated Teffs, which leads to autoimmunity.

Tregs is indeed sufficient to cause the inflammatory phenotype. In the adoptive transfer experiment, only very few Tregs are sufficient to rescue the autoimmune phenotype raising the possibility that MALT1^{C472A/C472A} Tregs may be less functional. In addition, the increase in IFN- γ and IL-4 production in MALT1 protease mutant mice may also indicate that deregulated T-cell effector functions could also be involved in the early onset of gastritis, which is no longer counteracted due to the loss of peripheral T-cell tolerance. In this respect, it remains an unresolved question why the autoimmune phenotype is manifested as gastritis. One can speculate that the microenvironment of the gastric

mucosa may support the onset of the disease, but it would be interesting if autoimmune reactions would further spread if the mice would survive beyond 15 weeks of age.

One obvious question is whether MALT1 protease activity is still a promising drug target for immunological diseases and in oncology. Jaworski *et al* show that MALT1^{C472A/C472A} are protected from the induction of EAE and colitis, lending strong support to a possible clinical use of MALT1 protease inhibitors in the treatment of autoimmune diseases. In line with this, the MALT1 inhibitor mepazine was recently shown to attenuate EAE severity in mice

(Mc Guire *et al*, 2014). However, it obviously needs to be evaluated whether therapeutic MALT1 inhibition could lead to a spontaneous autoimmune phenotype as observed in MALT1^{C472A/C472A} mice. Administration of the MALT1 inhibitor mepazine in an acute EAE model did not affect the number of Tregs (Mc Guire *et al*, 2014). Even though more prolonged treatment protocols and the usage of potentially more effective MALT1 inhibitors will be required to come to a definite conclusion, the data indicate that a permanent genetic inactivation of MALT1 protease may not reflect the situation of MALT1 pharmacological inhibition. First, the strength of MALT1 inhibition may be quite different in a genetic model or upon inhibitor treatment. Second, in contrast to a MALT1 protease inhibitor that prevents active site accessibility and thus substrate recognition, an active site mutant may still bind substrates and could exert additional effects. Although Jaworski *et al* did not report on any dominant-negative effects of MALT1 C472A in heterozygous animals, a mouse model that prevents protease activation instead of destroying the cleavage site may more closely reflect the situation of a pharmacological inhibitor. This could be achieved, for example, by mutating the ubiquitin-acceptor site K644 that is required for MALT1 proteolytic activation (Pelzer *et al*, 2013). Third and most important, the mouse model abrogated MALT1 activity already in early embryogenesis before the immune system and peripheral tolerance have developed. Thus, inactivation in adult mice by an inducible system will be more reminiscent to MALT1 inhibitor treatment. Further, generation of conditional transgenic mice will be required to establish the role of MALT1 protease in Treg biology.

Besides its physiological role in the immune system, MALT1 function has been studied in the context of hematological malignancies and especially ABC DLBCL biology. The first small-molecule MALT1 paracaspase inhibitors have been identified that effectively kill ABC DLBCL tumors in a murine xenotransplantation model (Fontan *et al*, 2012; Nagel *et al*, 2012). As this tumor model required the usage of immune-deficient mice, effects on the immune compartment were not analyzed. However, higher numbers of Foxp3-positive cells correlated with an adverse clinical outcome in DLBCL cohort enriched for ABC DLBCL

(Tzankov *et al*, 2008). Thus, in contrast to autoimmunity, inhibition of peripheral tolerance may even foster immune surveillance in lymphomas.

Taken together, the study by Jaworski *et al* underscores that deciphering the exact mechanism of autoimmune protection versus induction will be relevant for therapeutic targeting of the MALT1 protease in the context of autoimmune diseases as well as lymphomas.

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