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Commentary

New insights into cathelicidin modulation of adaptive immunity

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Cathelicidins are a family of host defence peptides that are known to selectively alter innate immunity in response to infection and other changes in immune status. A study in this issue of the *European Journal of Immunology* elucidates a new role for mouse cathelin-related antimicrobial peptide in the adaptive immune response by clearly demonstrating for the first time that a cathelicidin can alter T-cell-dependent activation of the humoral response in vivo and thus modulate the activities of both B and T lymphocytes.

Key words: B cells · Immune regulation · T cells

See accompanying article by Kin et al.

Previous work has demonstrated that a structurally diverse group of cationic amphipathic peptides, variously termed antimicrobial or host defence peptides (HDPs), can show direct antimicrobial activity that is reduced in vivo and in vitro when normal physiological levels of salinity and serum are present [1–5]. Under the same conditions, HDPs demonstrate profound and broad immunomodulatory activities including the selective modulation of innate immunity/inflammation (promoting anti-infective activity through cell recruitment and differentiation, while suppressing potentially damaging pro-inflammatory responses), enhancement of angiogenesis and wound healing, and adjuvant activity in augmenting and skewing adaptive immune responses [1–5]. Some of the most frequently studied HDPs are the cathelicidins, including human LL-37 and its rodent ortholog mouse cathelin-related antimicrobial peptide (mCRAMP). Cathelicidins are characterized by a conserved cathelin pro-domain located near the N-terminus that is removed as the peptide is secreted, leaving the active HDP [1, 5].

It is well known that cathelicidins and other HDPs influence adaptive immunity by acting on APCs (Fig. 1). Cathelicidins are secreted and taken up by macrophages, B cells, and DCs and their effects on these cells lead to selective immune activation [1, 2, 6]. Immature monocyte-derived DCs (MDDCs) transport LL-37 into the cytoplasm and nucleus, where LL-37 acts to upregulate CD86 and HLA-DR expression [7]. MDDCs derived in the presence of LL-37 also show various changes in surface expression including increased CD86 and CD11b in immature MDDCs [8]. These markers are associated with activation of the adaptive response; however, in response to Toll-like receptor (TLR) ligands, including lipopolysaccharide (LPS), cathelicidins can limit DC activation. For example, a model of allergic contact dermatitis found that wild-type mice had significantly decreased DC maturation and inflammation in response to LPS sensitization as compared with mice lacking mCRAMP [9]. Kandler et al. [10] found that LPS and other TLR ligands in combination with LL-37 led to a decrease in expression of HLA-DR, CD86, and other markers when applied to DCs. When such DCs were co-cultured with $CD4^+$ T cells, this reduced T-cell proliferation and their production of the T-cell activators IL-2 and IFN-γ [10]. Conversely, MDDCs derived with LL-37 in the culture medium showed normal maturation and increased CD11b and CD86 expression in response to LPS, and co-cultured T cells exposed to LPS and LL-37 had increased IFN-γ production but no significant change in cell proliferation [8], consistent with the concept that HDPs modulate rather than suppress or stimulate immune responses.

Other APCs include the M1 and M2 macrophages, polarized to a pro- and anti-inflammatory response, respectively. M1 macrophages promote the maturation of naïve $CD4^+$ T cells into Th1

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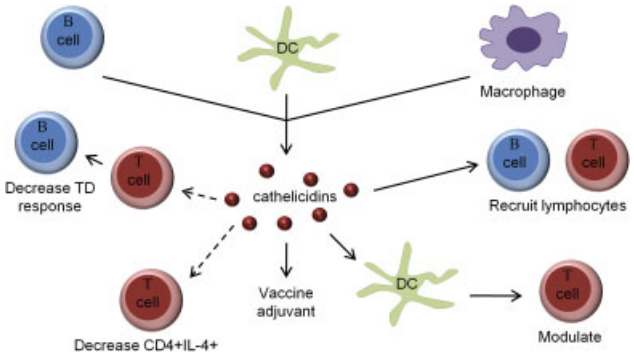

Figure 1. Cathelicidins impact adaptive immunity in multiple ways. Cathelicidins are released by APCs and lead to lymphocyte recruitment and DC-mediated T-cell modulation. They also act as useful vaccine adjuvants. The dashed arrows indicate functions from the work of Kin et al. [17] in this issue of the *European Journal of Immunology*, namely the roles of cathelicidins in T-cell-dependent responses and $CD4^+IL\text{-}4^+$ T-cell production.

cells, leading to activation of cell-mediated immunity, whereas M2 macrophages promote the development of Th2 cells and the humoral response. Both M1 and M2 macrophages show decreased TNF-α production in response to LL-37 [11], but LL-37 has also been demonstrated to make M2 macrophages more pro-inflammatory [12]. Together, these studies show that immune responses to cathelicidins depend on when the cathelicidin is applied and the presence of other signaling molecules such as TLR ligands.

While cathelicidins clearly influence APCs and their interactions with adaptive immune cells, evidence is emerging that cathelicidins have a more direct influence on the adaptive response. mCRAMP increased leukocyte recruitment, including lymphocytes, in a mouse air pouch model [13], and in vitro it was demonstrated that LL-37 significantly increased migration of $CD4^+$ but not $CD8^+$ lymphocytes [6]. B and T cells also showed altered secretion of cytokines and chemokines after LL-37 and LPS treatment compared with LPS alone [14]. In B cells, LL-37 limited class switching and cell proliferation after LPS/IFN-γ treatment [15]. Immunizing mice with OVA and mCRAMP led to an increase in specific anti-OVA IgG as compared with immunization with OVA alone [13], while a fusion of LL-37 and $M\text{-}CSFR_{J6\text{-}1}$ improved the specific immune response to tumors in mice [16]. The extent to which these responses are influenced by APCs and innate immunity is still unclear and many aspects of the relationship between cathelicidins and the adaptive response are largely unknown. Additionally, most in vivo studies have focused on injecting cathelicidin into rodents instead of examining its endogenous effects on adaptive immunity.

A study by Kin et al. [17] in this issue of the *European Journal of Immunology* brings new understanding to the role of cathelicidins in adaptive immunity by isolating populations of B and T cells from peritoneal lavage and the spleen in WT and $Camp^{-/-}$ mice lacking the gene for mCRAMP. Intriguingly, it was found that the response to, and expression of, IL-4 was altered in the $Camp^{-/-}$ mice and this affected both T and B cells. IL-4 is a key regulator of adaptive immunity that leads to an increased humoral response by promoting Th2 cell development [18]. Under IL-4-induced Th2 conditions, IL-4 was significantly increased in the $Camp^{-/-}$ T cells and the expression was reduced to WT levels when mCRAMP was added. In contrast, $CD4^+$ T cells from $Camp^{-/-}$ mice showed a similar expression of IFN-γ as WT $CD4^+$ T cells when both were cultured under IFN-γ-induced Th1 conditions [17]. IL-4 also enhances class switching in B cells, increasing IgG1 and IgE expression in mice [19]. In the Kin et al. study [17], B cells isolated from WT and $Camp^{-/-}$ mice showed no differences in IgM and IgG3 expression when cultured with LPS, or in IgG2c levels when CD40L/IFN-γ was used as a stimulus. Surprisingly, when the B cells were cultured with CD40L/IL-4, the $Camp^{-/-}$ cells showed decreased IgG1 and IgE expression. The antibody levels were restored to those of WT cells when mCRAMP was included in the culture conditions. The decreased IgG1 production was determined to be from reduced mRNA expression rather than changes in class switching.

Kin et al. [17] further demonstrated a relationship between mCRAMP and B and T cells by injecting mice with type 1 and 2 antigens or T-cell-dependent antigens [17]. T-cell-dependent antigens require Th2 cells to activate B cells and produce antibody, whereas type 1 and 2 antigens are T-cell independent and do not require a Th2 signal. While the T-cell-independent responses were similar between WT and $Camp^{-/-}$ mice, the T-cell-dependent responses using TNP-OVA/alum led to significantly more TNP-OVA-specific IgG1 antibodies in the $Camp^{-/-}$ mice. Consistent with this, splenocytes from $Camp^{-/-}$ mice that had been administered with a T-cell-dependent antigen were also found to have increased IL-4 mRNA expression and increased numbers of $CD4^+IL\text{-}4^+$ T cells as compared with those from similarly treated WT mice.

The connections between mCRAMP and IL-4 open up intriguing possibilities for the role of cathelicidins in adaptive immunity. In the mice given TNP-OVA/alum and in the in vitro T cells, the responses indicate that mCRAMP suppresses both the development of a Th2 response and the Th2-mediated class switching to IgG1 through IL-4 [17, 19]. In contrast, the results from isolated B cells stimulated with CD40L/IL-4 indicated that mCRAMP promoted IgG1 production by increasing transcription [17]. Kurosaka et al. [13] showed that mCRAMP administered as an adjuvant with OVA increases IL-4 and OVA-specific IgG1 in splenocytes, although the response was not Th2-mediated. Similarly, An et al. [16] found that LL-37 acts as an effective adjuvant in a vaccine against tumor cells, while Davidson et al. [8] found a bias towards a Th1 response in human DCs. The conflicting reports may reflect methodological differences, such as using $Camp^{-/-}$ mice versus injecting cathelicidin into WT mice, or the timing and nature of other stimuli applied. Nonetheless, these studies indicate that mCRAMP likely mediates its effects on adaptive immunity through many other factors in addition to IL-4.

The work by Kin et al. [17] shows that mCRAMP alters B- and T-cell responses, highlighting the novel role of mCRAMP in the T-cell-dependent activation of B cells, and thus providing

evidence that mCRAMP and other cathelicidins have a greater role in the adaptive immune response than previously appreciated. However, many questions still remain, particularly whether mCRAMP acts directly on components of the adaptive immune system or if intermediates are involved. It is also of interest to determine whether the changes seen by Kin et al. [17] in response to T-cell-dependent antigen are due to mCRAMP altering both T and B cells or whether only one cell type is directly involved. The use of conditional knockouts or adoptive transfer to examine when *Camp* is absent from either T or B cells will help resolve these issues. Similar models could also be used to clarify the functions of APCs in shaping the $Camp^{-/-}$ effects on lymphocytes. Determining the specific cells and pathways altered by mCRAMP will provide further insight into the roles of cathelicidins in bridging innate and adaptive immunity.

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Abbreviations: **HDPs:** host defence peptides · **mCRAMP:** mouse cathelin-related antimicrobial peptide

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