

Precision attack on calcineurin in macrophages: a new anti-inflammatory weapon

Joachim L Schultze

Inflammation is a hallmark of many common diseases ranging from arthritis, atherosclerosis, or obesity to Alzheimer's disease and cancer. Identifying anti-inflammatory mechanisms is therefore an important and timely task of modern biomedicine. In this issue of *The EMBO Journal*, a study conducted by Escolano and colleagues target a particular interaction site of calcineurin with NFAT in macrophages to elicit profound anti-inflammatory effects (Escolano *et al*, 2014).

See also: **A Escolano *et al*** (May 2014)

There is increasing evidence that major diseases including atherosclerosis, diabetes, obesity and similarly arthritis, allergies and even Alzheimer's and certain cancers are associated with inflammation. The type of inflammation associated with these common diseases is the so-called sterile inflammatory response caused by the recognition of a variety of irritant particles, including crystals, minerals, protein aggregates or cellular debris (Rock *et al*, 2010). Macrophages are key players among innate immune cells sensing such patterns and are indeed involved in all diseases mentioned above (Wynn *et al*, 2013). It is therefore not surprising that one of the major therapeutic goals is to target inflammation to at least alleviate symptoms but probably even to cure some of these illnesses. There are several classes of drugs that possess anti-inflammatory effects such as steroids and non-steroidal anti-inflammatory drugs (NSAIDs). Some anti-inflammatory drugs including the steroids but more importantly

the calcineurin (CN) inhibitors (CNIs) cyclosporine (CsA), FK506 or rapamycin are not only anti-inflammatory, but also potent immunosuppressive (IS) drugs. This class of molecules has been tailored mainly toward T-cell-mediated pathologies as seen in transplantation (Ponticelli, 2011) and therefore has not been considered for many of the common diseases where innate immune cells seem to play a major role in the inflammatory response. Moreover, their well-known and severe side effects have limited their broad use under less life-threatening conditions.

A novel study conducted by Escolano *et al* (2014) presents a new strategy to suppress inflammation while revisiting calcineurin as specific, molecular target. In previous work, Juan Miguel Redondo and his co-workers had introduced a peptide that interferes with one of the two CN-binding motifs of NFAT (the so-called LxVP peptide). In contrast to other CNIs, the LxVP peptide also inhibits the phosphatase activity of CN (Martinez-Martinez *et al*, 2006; Rodríguez *et al*, 2009). In their new report, they demonstrate that macrophages with constitutive or inducible CN deficiency, but not treatment with classical IS drugs such as CsA or FK506, confer anti-inflammatory properties to macrophages (Fig 1). Moreover, transfer of such macrophages protected mice from collagen-induced arthritis (CIA), a murine model of joint inflammation. Clinically more important, Escolano *et al* could demonstrate that the expression of an LxVP peptide interfering with the binding of CN to NFAT phenocopied the genetic

deletion of CN, indicating that suppression of CN activity in macrophages mediates a clinically relevant anti-inflammatory effect *in vivo*. LxVP peptide-mediated CN blockade triggered the release of p38 MAPK from MKP1-mediated repression, which was not seen when CsA or FK506 were used at physiologically relevant concentrations, clearly pointing to mechanistic differences between these CNIs. In a series of experiments, Escolano *et al* demonstrated that the LxVP peptide could be effectively delivered via systemic or local lentiviral gene transfer to treat distinct inflammatory reactions including CIA, but also zymosan-induced acute paw inflammation or oxazolone-induced contact hypersensitivity.

The findings presented by Escolano *et al* open up new avenues targeting macrophages to repress the inflammatory responses seen in many common diseases. However, before this approach can be applied to patients, important questions need to be answered: For example, what is the relationship of anti-inflammatory macrophages induced by the LxVP peptide with other anti-inflammatory macrophages? Global transcriptome or proteome analyses would allow addressing this question in a comprehensive fashion (Xue *et al*, 2014). Such data might also be used to determine major downstream effector mechanisms that execute calcineurin blockade by the LxVP peptide. Since there seem to be significant differences between the LxVP peptide and other inhibitors of calcineurin, global assessment of changes in gene expression in response to these different

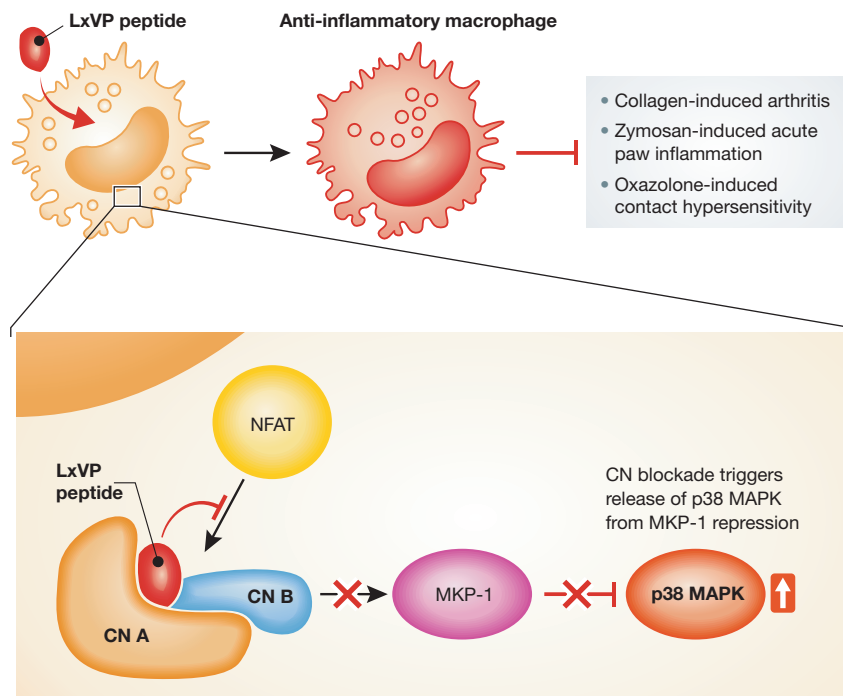


Figure 1. In macrophages, the LxVP peptide can inhibit the interaction of calcineurin (CN A and CN B) with NFAT.

This blockade leads to reduced activity of MKP-1, a dual specificity protein phosphatase. Reduced activity of MKP1 itself results in increased activity of p38 MAPK, which is associated with reprogramming of macrophages to become anti-inflammatory. In murine *in vivo* models, these anti-inflammatory macrophages inhibit the inflammatory model diseases: collagen-induced arthritis, zymosan-induced acute paw inflammation and oxazolone-induced contact hypersensitivity.

inhibitors might quickly lead to an understanding of the profound differences in anti-inflammatory properties in macrophages. This area of research needs further exploration, particularly since previous work with CsA and FK506 (Kang *et al*, 2007) did similarly not reveal the molecular basis of this intriguing phenomenon. Moreover, such genome-wide data might be utilized to identify specific markers of these cells that could serve as biomarkers to monitor the efficacy of LxVP peptide-based therapeutic approaches *in vivo*. Another issue concerns the translation of these findings to a clinical setting. Escolano *et al* suggest cell therapy with CN-gene-

deleted or LxVP-inhibited macrophages or alternatively the use of lentiviruses for delivery of the LxVP peptide-encoding RNA. However, a specific blockade of calcineurin in macrophages directly *in vivo* by a chemical compound might be a much more appealing approach, particularly to be picked up by biotech or the pharmaceutical industry. The seemingly higher specificity of the LxVP peptide among the CNIs and the novel approach to directly target macrophages warrant further investigation into its possible benefits in the context of a variety of diseases that are known to present with macrophage-associated or macrophage-mediated inflammation.

Conflict of interest

The author declares that he has no conflict of interest.

References

- Escolano A, Martínez-Martínez S, Alfranca A, Urso K, Izquierdo HM, Delgado M, Martín F, Sabio G, Sancho D, Gómez-del Arco P, Redondo JM (2014) Specific calcineurin-targeting in macrophages confers resistance to inflammation via MKP-1 and p38. *EMBO J* 33: 1117–1133
- Kang YJ, Kusler B, Otsuka M, Hughes M, Suzuki N, Suzuki S, Yeh WC, Akira S, Han J, Jones PP (2007) Calcineurin negatively regulates TLR-mediated activation pathways. *J Immunol* 179: 4598–4607
- Martínez-Martínez S, Rodríguez A, López-Maderuelo MD, Ortega-Pérez I, Vázquez J, Redondo JM (2006) Blockade of NFAT activation by the second calcineurin binding site. *J Biol Chem* 281: 6227–6235
- Ponticelli C (2011) Present and future of immunosuppressive therapy in kidney transplantation. *Transplant Proc* 43: 2439–2440
- Rock KL, Latz E, Ontiveros F, Kono H (2010) The sterile inflammatory response. *Ann Rev Immunol* 28: 321–342
- Rodríguez A, Roy J, Martínez-Martínez S, López-Maderuelo MD, Niño-Moreno P, Ortí L, Pantoja-Uceda D, Pineda-Lucena A, Cyert MS, Redondo JM (2009) A conserved docking surface on calcineurin mediates interaction with substrates and immunosuppressants. *Mol Cell* 33: 616–626
- Wynn TA, Chawla A, Pollard JW (2013) Macrophage biology in development, homeostasis and disease. *Nature* 496: 445–455
- Xue J, Schmidt SV, Sander J, Draffehn AM, Krebs W, Quester I, De Nardo D, Gohel TD, Emde M, Schmidleithner L, Ganesan H, Nino-Castro A, Mallmann M, Labzin L, Theis H, Kraut M, Beyer M, Latz E, Freeman TC, Ulas T *et al* (2014) Transcriptome-based network analysis reveals a spectrum model of human macrophage activation. *Immunity* 40: 274–288