European Journal of Immunology
Highlights

COMMENTARY:

A new role for CCR5 in innate immunity – binding to bacterial heat shock protein 70

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CCR5 has been associated with adaptive immune responses, because of its expression on effector and memory T cells, and dentric cells. In this issue of the *European Journal of Immunology*, Whittall *et al.* identify CCR5 as a receptor that binds bacterial heat shock protein 70 (HSP70). Thus HSP70 may stimulate responses by CCR5$^+$ T cells or dendritic cells, particularly at epithelial surfaces. These findings imply a role for CCR5 in innate immunity, in addition to its more established role as a chemoattractant receptor for adaptive immune responses.

Received 31/7/06
Accepted 4/8/06

[DOI 10.1002/eji.200636551]

Key words: CCR5 · HSP70 · Innate Immunity · Infection

See accompanying article http:dx.doi.org/10.1002/eji.200635953

CCR5 is one of those molecules that grows more and more fascinating with time. Now, in this issue of the *European Journal of Immunology*, Whittall *et al.* [1] define a new role for CCR5 as a receptor that binds bacterial heat shock protein 70 (HSP70).

CCR5 shot to prominence in the mid 1990s with the discovery that particular strains of HIV-1 used this and other chemokine receptors as entry portals for infection of macrophages and T cells. Then came the important discovery that some individuals, particularly of European descent lacked surface expression of CCR5 because of a 32 base pair deletion in the coding sequence, and this afforded protection from HIV-1 infection [2]. A logical assumption at that time was that HIV-1 or a similar virus led to genetic selection for this mutation by conferring a survival advantage on Δ32 homozygous individuals; however Δ32 is not the only genetic element that influences CCR5 expression, and indeed numerous polymorphisms within the CCR5 promoter and coding sequence likewise affect expression levels. In our studies with anti-CCR5 mAbs, we noted considerable variation from person to person in CCR5 expression among non-Δ32 individuals [3], which relates to these polymorphisms. The connotation is that CCR5 is a molecule that is crucial for immune responses to microbial pathogens. In addition, one of the high affinity ligands of CCR5, CCL3L1, a close relative of CCL3 (MIP-1α) shows significant differences between ethnic groups [4]. Thus it appears that, for whatever reason, evolution has been tweaking expression of CCR5 and its ligands amongst different populations to meet environmental challenges.

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Pathogens that may have influenced the genetics of CCR5 or its ligands in different ethnic groups may be entities other than HIV-1, or even viruses. The Δ32 deletion almost certainly evolved by selection in response to pathogens other than HIV-1, because CCR5 Δ32 emerged at least 700 years ago [5] or even longer [6]; bubonic plague caused by the enterobacterium *Yersinia pestis* has been suggested as an epidemic that might have selected for this mutation. An interesting question is whether genetic variations in primate or human CCR5 and its ligands emerged as protective mutations against CCR5-binding pathogens such as HIV-1, or whether CCR5 simply serves as an immune response gene product that affects the nature or magnitude of the immune response through effects on leukocyte recruitment. CCR5 is expressed by T cells, especially effector/memory type T cells, and Th1 T cells, and also by macrophages and dendritic cells (DCs) [3, 7]. CCR5 is especially prominent on T cells at epithelial surfaces [3], and likely facilitates peripheral tissue homing by T cells to sites where pathogens are often encountered, such as epithelial surfaces of the gut or genital tract. For a while the functional relevance of CCR5 remained obscure as no phenotype was reported for CCR5-deficient mice; however, recently these mice have been shown to be susceptible to West Nile virus [8], establishing CCR5 as an important anti-viral element that acts by regulating leukocyte traffic, in this case to the brain.

The reason why Whittall *et al.* [1] became alerted to CCR5 as a receptor for HSP70 was that they discovered an inhibitory effect of HSP70 on HIV-1 infectivity. HSP70 is a member of a family of molecular chaperones that facilitate protein synthesis, folding, and translocation [9]. *Mycobacterium tuberculosis*-derived HSP70 is known to function as an adjuvant in stimulating host immune responses. The group of Thomas Lehner previously established that HSP70 stimulated production of cytokines and chemokines by leukocytes [10]. These included CCL3 (MIP-1α) and CCL4 (RANTES) [10], CCR5 ligands which might serve to attract appropriate immune cells and enhance responses. This microbial HSP70 stimulation of chemokine production by leukocytes occurs through binding of HSP70 to surface expressed CD40 [10]. The question was, did HSP70 inhibit HIV infection solely through its ability to stimulate CCR5-binding chemokines upon CD40 stimulation, or did HSP70 interfere directly with HIV-1 entry, by physically binding to CCR5, in the same way that bacterial HSP70 binds directly to CD40 [10]?

Whittal *et al.* [1] used a variety of approaches and showed that CCR5 did in fact appear to function as a receptor for HSP70. Labeled HSP70 bound in a dose-dependent manner to CCR5 transfected cell lines and this could be blocked with a CCR5 small molecule inhibitor, TAK 779. Moreover pertussis toxin, an inhibitor of Gαi protein signaling, inhibited cytokine production by HSP70-stimulated primary human DCs. Activation of HEK 293 cells transfected with CCR5 elicited calcium signaling by HSP70, and TAK 779 inhibited this. Furthermore, HSP70 stimulated significant p38 MAP kinase phosphorylation in CCR5 transfected but not untransfected HEK 293 cells. MAP kinases are important signaling molecules for chemoattractant receptor function, as well as many other processes in immune cells. Thus HSP70-mediated signaling through CCR5 may provide signals additional to those occurring through other receptor systems. Finally, surface plasmon resonance (Biacore) showed that HSP70 specifically bound to peptides derived from CCR5, particularly regions from the N-terminus and second extracellular loop. The N-terminus of CCR5 is particularly important for the binding and internalization of R5 strains of HIV-1, while the second extracellular loop is critical for chemokine binding [11, 12].

What could be the purpose of HSP70 stimulation of $CCR5^+$ cells? HSP70 from bacteria could provide innate signals, to stimulate or co-stimulate CCR5 expressing cells — T cells, macrophages or DCs. This may provide an added boost to the response of $CCR5^+$ cells at epithelial surfaces. CCR5 is expressed by "immature" DC, which migrate to inflamed peripheral tissues where they capture antigens [13]. Following maturation through stimulation with bacterial products such as LPS, they up-regulate CCR7 and migrate to lymph nodes where they assume new functions such as stimulation of naive T cells. It is conceivable that bacterial HSP70 serves as a chemoattractant for $CCR5^+$ cells, and might also affect DC maturation. It will be interesting to see whether other chemokine receptors bind bacterial HSP, and understand whether this is truly by design, to aid immune responses to bacteria, or whether HSP has some sort of subversive activity in immune cell recruitment. Regardless, the interaction of CCR5 with bacterial HSP70 elevates the status of CCR5 and its ligands to crucial molecules that operate at the very core of immunity to microbial pathogens.

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