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S.Bradbrook/NPG

CHEMOKINES

Neutrophils leave a trail for T cells

> membrane trails function as a slow-release depot for CXCL12

The recruitment of activated effector T cells to sites of infection is mediated by chemokines, which are produced during the recruitment of innate immune cells, such as neutrophils, to the infected tissue. This paper describes how neutrophil and T cell migration are linked during influenza virus infection by showing that early-recruited neutrophils in the trachea leave behind a long-lasting chemokine trail.

In C57BL/6 mice infected with influenza A virus, there is a rapid, transient infiltration of neutrophils in the trachea peaking on day 4 post-infection, followed by $CD8^+$ T cell recruitment on days 6–8. When neutrophils were depleted using a LY6G-specific antibody, the primary $CD8^+$ T cell response in the trachea was decreased, virus clearance was delayed, and the number of memory T cells remaining after virus clearance was reduced. By contrast, neutrophil depletion did not affect the number of influenza virus-specific T cells in draining lymph nodes.

Therefore, neutrophil depletion in this model inhibits T cell responses in the tissue without affecting T cell priming in lymph nodes, which suggests an effect on T cell homing to the tissue. $CD8^+$ T cells were present only in the subepithelium of infected tracheae in control mice, whereas they remained in the interstitium and more distal to the epithelium after neutrophil depletion, as observed by whole-mount immunostaining. The inhibited migration of virus-specific T cells to the tracheal epithelium after neutrophil depletion was also observed using intravital two-photon microscopy.

Further studies indicated that chemokine signals involving CXC-chemokine ligand 12 (CXCL12) link the neutrophil and T cell responses in the trachea during influenza virus infection. Treatment with an antagonist of CXC-chemokine receptor 4 (CXCR4), which is the receptor for CXCL12 and is expressed by T cells, had the same effect as neutrophil depletion on $CD8^+$ T cell recruitment to the tracheal epithelium. The importance of local neutrophil-derived CXCL12 for T cell recruitment was confirmed in mice with a granulocyte-specific conditional depletion of CXCL12, which induced a delayed recruitment of $CD8^+$ T cells to infected trachea and delayed virus clearance.

In vitro, the migration of activated $CD8^+$ T cells was significantly enhanced on coverslips previously exposed to migrating neutrophils and then washed compared with control coverslips, and this migration was abolished by adding the CXCR4 antagonist. No neutrophil cell bodies could be detected on the washed coverslips, but a large number of membrane particles were present. These membrane particles were shown to be left behind by the long membrane tethers that form during neutrophil migration, and of more than 50 cytokines and chemokines that were screened, only CXCL12 was enriched in these neutrophil trails. Coverslips pre-exposed to CXCL12-deficient migrating neutrophils did not increase T cell migration despite the presence of neutrophil trails.

The authors suggest that the membrane trails function as a slow-release depot for CXCL12, which would otherwise immediately diffuse away from the site of neutrophil production and/or be rapidly degraded. Indeed, the neutrophil trails could attract T cells over a distance of more than 500 μm in a microchamber chemotaxis assay, which suggests that they release soluble CXCL12. In support of their long-lasting effects, neutrophil trails containing CXCL12 were observed in influenza virus-infected trachea *in vivo* and remained after the neutrophils were cleared from the tissue.

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ORIGINAL RESEARCH PAPER Lim, K. *et al.* Neutrophil trails guide influenza-specific $CD8^+$ T cells in the airways. *Science* **349**, aaa4352 (2015).