# CD8+ T cell Bystander Activation Contributes to Early Infection Control

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The immune response is generally divided into two distinct phases: an early innate response, which recognizes broad groups of pathogens based on shared molecular patterns, and a later adaptive response which potently and specifically targets individual pathogens through recognition of specific antigens. However, it has been recognized that memory CD8+ T cells, which play a role in the adaptive response, may also be activated non-specifically through a process called bystander activation. CD8+ memory T cells accumulate with age, and therefore may play an increasingly important role in the immune response over time. However, the importance of bystander activation during the course of infection is unclear. In a recent study published in Cell Reports, Talyn Chu and Dr. Martin Prlic (Vaccine and Infectious Disease Division), along with an international team of collaborators, demonstrate that the bystander effect is induced by exposure to inflammatory cytokines, and that this effect is important for controlling pathogens during the early stages of infection.

Bystander activation is the phenomenon of non-antigen-specific CD8+ T cells being activated by inflammation rather than an antigen. These bystander activated T cells (BA-CTLs) produce both interferon γ, which induces both innate and adaptive immune responses, and granzyme B, an effector molecule that induces apoptosis. While recent studies have demonstrated that both monocytes and dendritic cells promote bystander activation, the mechanism behind this activation is unclear.

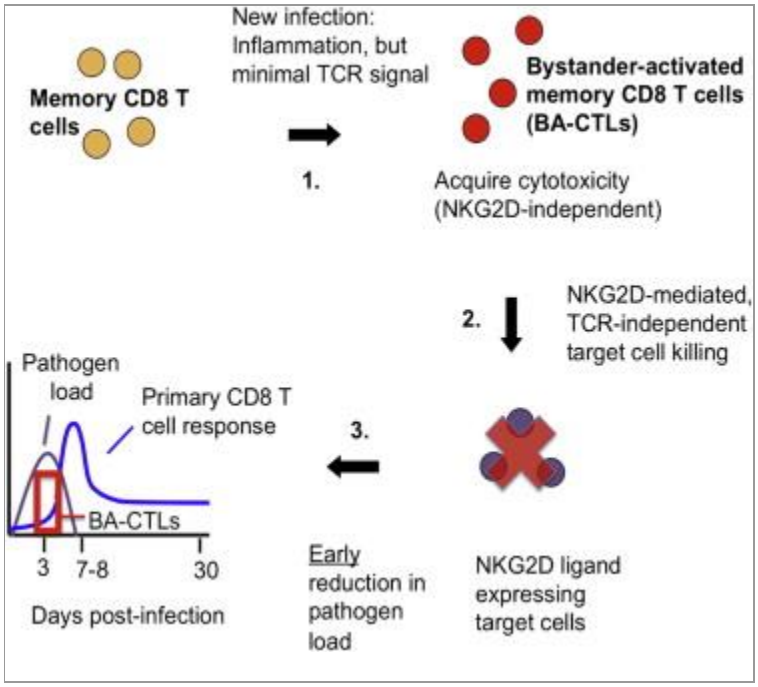
To phenotype BA-CTLs, the authors transferred naïve T cells specific for a well-characterized ovalbumin antigen into mice. The mice were infected with virus expressing this antigen, and the adoptively transferred T cells responded to the antigen, acquiring a memory phenotype. The memory CD8+ T cells also expressed NKG2D, a receptor which binds to several stress-induced ligands on the cell surface. This receptor was not present on naïve T cells. 30 days later the mice were challenged with Listeria monocytogenes (LM), a bacterium which does not express the ovalbumin antigen, and should not stimulate the TCR of the adoptively transferred T cells. Approximately half of the NKG2D+ cells expressed granzyme B after LM challenge, confirming a BA-CTL phenotype.

To determine whether or not TCR interactions were required for bystander activation, the authors employed a mouse model which expresses GFP after TCR stimulation, but not after cytokine stimulation. The team found that BA-CTLs do receive weak TCR signaling, but at levels below direct TCR binding in naïve cells. To determine which signals directly stimulate the effector function of the BA-CTLs, the authors exposed naïve and memory CD8+ T cells to IL-12, IL-15, and IL-18. Granzyme B expression increased in the memory population, but not the naïve cells, after only 6 hours. However, NKG2D expression increased only slightly. Taken together, these data suggest inflammatory cytokines can directly induce BA-CTL activity in memory CD8+ T cells, but do not regulate NKG2D expression.

The authors next confirmed that BA-CTLs can directly kill infected and TLR-stimulated cells expressing the NKG2D ligand Rae-1 on their cell surface, although this effect was less efficient than inducing cell death through classical TCR-mediated CD8+ T cell cytotoxicity. Finally, the team tested the role of BA-CTL during an in vivo infection. Mice were injected with an antibody blocking NKG2D two days before and after challenge with LM. BA-CTLs still expressed granzyme B whether or not NKG2D was blocked; however, NKG2D-blocked animals had more than 10-times as many bacteria five days post-infection. These data suggest that effector phenotypes, as represented by granzyme B expression, may be acquired in vivo without NKG2D stimulation; however, NKG2D is critical for target cell identification.

BA-CTLs recognize infected cells in an innate-like manner prior to the onset of de novo adaptive responses. Taken together, these data suggest that BA-CTLs play a crucial role in pathogen control during the early stages of infection.
This innate-like role for memory CD8 T cells may be of great importance in the elderly, as the distribution of naïve and memory T cell populations changes with increasing age and T cells with a memory phenotype accumulate. “We are now examining the consequences of bystander-activation following vaccination. Our current (unpublished) data suggest that bystander-activated CD8 T cells limit antigen-availability in a vaccine scenario thus decreasing the immune response. We are testing this model now and hope that this next step will provide much needed insight on how to improve vaccine efficacy in the elderly population,” said Dr. Prlic.

Chu T, Tyznik AJ, Roepke S, Berkley AM, Woodward-Davis A, Pattacini L, Bevan MJ, Zehn D, Prlic M. 2013.Bystander-activated memory CD8 T cells control early pathogen load in an innate-like, NKG2D-dependent manner. *Cell Reports.* 3(3):701-8.

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Schematic of bystander-activated cytotoxicity demonstrating 1) initiation of the BA-CTL response through inflammatory cytokines and modest T-cell receptor signaling, which leads to 2) cytotoxicity mediated through NKG2D. This pathway results in 3) a significant reduction to early pathogen load in an antigen non-specific manner.