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Adjuvant-induced survival signaling in clonally expanded T cells is associated with transient increases in pAkt levels and sustained uptake of glucose

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Abstract

Immunological adjuvants help increase the number of T cells responding to an immunizing antigen. Part of the increase is due to promotion of survival of clonally expanded T cells in the face of waning antigen load and subsequent growth-factor withdrawal. The phosphatidylinositide-3 kinase (PI3-kinase)/Akt pathway is activated upon T cell stimulation and plays a critical role in clonal expansion by mediating several aspects of co-stimulation in a growth-factor-dependent manner. We hypothesized that adjuvants must either cause the PI3-kinase/Akt pathway to operate in the absence of growth-factor or to render T cells independent of continuous PI3-kinase signaling for their survival. To determine which is true, mice were treated with model antigen in the presence or absence of the natural adjuvant lipopolysaccharide (LPS). T cells from treated mice were assayed for their dependence on PI3-kinase signaling by measuring (i) levels of phosphorylated Akt, (ii) survival after culture in the presence of the PI3-kinase inhibitor LY294002, and (iii) the amount of glucose uptake upon ex vivo culture. The results show that although LPS treatment increased the induced PI3-kinase activity, the presence of PI3-kinase inhibitor did not affect glucose uptake or survival of T cells, an attribute the cells acquired within 4 h of LPS injection. Therefore, adjuvant-dependent survival effects do not require continuous PI3-kinase activity to occur, a finding that may explain how activated T cells survive antigen-withdrawal long enough to traffic from priming lymph nodes to sites of infection.

Keywords: Cytokine; Glucose; Hypoxia; LPS; LY294002; PI3-kinase/pAkt; Superantigen; T cells

Introduction

We seek to understand the mechanisms by which immunological adjuvants help keep activated T cells alive during an immune response. Survival pathways that operate early after T cell activation have been extensively studied using various mitogenic factors to stimulate division of cultured cells. Such studies have shown that T cells must be stimulated through both their T cell receptors and co-stimulatory molecules in order to divide (Mueller et al., 1989). However, in vitro conditions for T cell activation differ markedly from those in vivo, and the sufficiency of co-stimulation for long lasting T cell responses in animals has been questioned. For example, adjuvants must exert survival as well as co-stimulatory effects (Vella et al., 1995, 1997)

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but the precise nature of adjuvant-induced survival signaling remains ill-defined. Phosphatidylinositide-3 kinase (PI3-kinase) through one of its key substrates, Akt, has been described as playing several co-stimulatory and anti-apoptotic roles in T cells (Parry et al., 1997; Appleman et al., 2002; Okkenhaug et al., 2004). Therefore, we tested T cells for their dependence on PI3-kinase during various stages of clonal expansion in vivo in order to test the idea that adjuvants render the cells independent of PI3-kinase for survival at some point during clonal expansion.

One of the chief differences between in vitro and in vivo models of T cell proliferation is that, as cells divide in vivo they move away from the antigen-presenting cells that stimulated them (Catron et al., 2004), while in vitro the activated cells remain in a growth-factor-rich environment. Migration of recently divided cells away from lymphoid centers of antigen and cytokine exposure means that activated T cells must survive growth-factor withdrawal in order to traffic to sites of immune defense. Because growth-factor-dependent survival has been closely linked to PI3-kinase signaling, the fact that adjuvants cause T cells to survive growth-factor withdrawal suggests that either PI3-kinase activity becomes independent of the presence of growth-factor or that PI3-kinase signaling ceases to be needed for the cells to persist. The involvement of PI3-kinase/Akt signaling in T cell survival early during clonal expansion includes enhancement of proliferation and short-term survival through increased expression of cytokines (Kane et al., 1999, 2001; Lali et al., 2004) and of anti-apoptotic protein Bcl2 (Adachi et al., 1998), which we have found is induced and then lost from T cells upon activation (Mitchell et al., 2002). Signaling through phosphorylated Akt (pAkt) also increases production of anti-apoptotic Bcl-X_L, although its increased expression is also transient (Akbar et al., 1996; Madrid et al., 2000; Burr et al., 2001; Mitchell et al., 2002). pAkt has also been reported to phosphorylate GSK3β (Shaw et al., 1997; Gold et al., 2000) and Forkhead transcription factors (Brunet et al., 1999; Plas and Thompson., 2003) in activated T cells which inactivates their pro-apoptotic activities. Other important properties of pAkt include the maintenance of nutrient and energy sources for activated T cells, through increased uptake of glucose and maintenance of size and viability of these cells in tissue culture experiments (Frauwirth et al., 2002; Rathmell et al., 2003a, b). Most of these experimental observations are restricted to a short time period, within a few hours after the activation of cells with antigen or mitotic stimuli in tissue culture.

In vivo, activated T cells rapidly lose antigen signaling and are therefore eliminated due to cytokine or growth-factor withdrawal (Mitchell et al., 2002). However, we know that a portion of these activated T cells survives in spite of factor withdrawal and that this survival is adjuvant dependent. For example, recent reports suggest that the death of antigen-activated T cells upon growth-factor withdrawal is avoided with the addition of signals generated by the engagement of Toll-like receptors (TLRs) on antigen presenting cells (APCs) by microbial products (Vella et al., 1995, 1997). The TLRs bind different pathogen-associated molecules such as bacterial lipopolysaccharides (LPS) and, through associated APC, send a series of signals to the T cells to boost their responses. These responses include increased clonal expansion and maintenance of pathogen-specific T cells, which results in effective immunity (Vella et al., 1995; Mitchell et al., 2001, 2002). Hence, TLRs are considered to be the 'adjuvant receptors' of the immune system (Medzhitov and Janeway, 2000).

Analysis of vaccinia virus-infected mice, in which the adjuvant effects of bystander infection were studied, showed that IL-2 and other common γ-chain receptor family members were not associated with or needed for adjuvant effects on T cell survival (Mitchell et al., 1999). Similar results were observed when using the TLR-4 agonist LPS as adjuvant (Mitchell et al., 2002). These patterns argue that adjuvants help T cells survive acute growth-factor withdrawal rather than preventing the withdrawal from happening. Since PI3-kinase/Akt signaling is closely connected to both cytokine exposure and activated T cell survival, keeping the cells alive during cytokine withdrawal means that either the PI3-kinase/Akt pathway becomes independent of cytokine, at least temporarily, or ceases to be needed altogether at later stages of clonal expansion. We found that adjuvant exposure prolonged activated T cell survival in the absence of continuous PI3-kinase signaling. Dependence on PI3-kinase signaling for survival began to decrease within 4 h of adjuvant injection and was characterized by increased glucose uptake by T cells that had reached peak clonal expansion. This conclusion has important implications for understanding how T cells survive the growth-factor withdrawal phase of clonal expansion.

Materials and methods

T cell activation, in vitro culture and survival analysis

Female B10.BR mice (The Jackson laboratories, Bar Harbor, ME) were maintained in a specific pathogen-free facility at the University of Louisville. CD4 T cells bearing Vβ3 as a part of their T cell receptor (TCR) were activated by injecting mice, via the tail vein, with 0.1 μg of the superantigen staphylococcal enterotoxin-A (SEA; Toxin Technologies, Sarasota, FL) and 16 h later, with 10 μg of LPS (from *Salmonella typhosa*; Sigma