Chapter 8
Adjuvant Induced Glucose Uptake by Activated T Cells is not Correlated with Increased Survival

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Abstract  Authors contributed equally to this manuscript Natural adjuvants, such as bacterial lipopolysaccharide (LPS), activate antigen presenting cells via Toll-like receptors and, indirectly, increase the survival of antigen-activated T cells. The molecular mechanisms leading to increased survival remain poorly defined. Because T cell clonal expansion leads to high energy demands, we hypothesized that increased glucose uptake and/or utilization in adjuvant-activated T cells could be important molecular event(s) that would lead to adjuvant-associated T cell survival advantage. Using a fluorescent analog of 2-deoxyglucose, 2-NBDG, we measured glucose accumulation and rate of uptake in T cells from mice treated with antigen in the absence or presence of LPS. Although adjuvant activated T cells increased the accumulation of 2-NBDG, the rate of uptake was unchanged compared to cells activated with only antigen. Furthermore, glucose transport inhibitors, cytochalasin B or phloretin, decreased the accumulation of glucose in adjuvant-treated T cells, but this decrease did not impair adjuvant-associated survival advantages. Together, these data indicate that increased glucose uptake through glucose transporters is not required for increased survival of activated T cells.

8.1 Introduction

T cell proliferation initiated by activation with antigen is followed by rapid death due to a loss of antigen exposure and acute shortage of pro-survival cytokine signals. This deletion, although important for reducing the risk of autoimmune responses, hampers immunity because complete elimination of the

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responding T cells would reduce the ability of a host to resist subsequent infection. Vella et al. first showed that in vivo activated T cells were protected from growth-factor withdrawal induced death by the natural adjuvant LPS [1]. The mechanism(s) by which natural adjuvants keep activated T cells alive in an immune response is still not fully understood. Co-stimulatory factors and known pro-survival molecules like Bcl2 and Bcl-XL were previously shown to be insufficient for adjuvant mediated survival [2, 3]. Activated T cell survival is often linked to the activation of PI3-kinase during clonal expansion because it enhances proliferation and short-term survival through increased expression of cytokines [4, 5]. We recently reported that although transiently activated by adjuvant exposure, PI3-kinase stimulation was insufficient to account for adjuvant-induced survival and suggested that induction of post PI3-kinase signaling effects must be involved [6].

PI3-kinase activity is reported to induce increased localization of the glucose transporter GLUT1 to the cell surface [7]. Recent reports also show that PI3-kinase stimulates increases in GLUT1 expression in B cells upon activation with antigen, which results in increased glucose uptake [8]. Increased uptake of glucose has also been reported to be required by activated T cells for maintenance of aerobic glycolysis, a metabolic system that is proposed to provide the carbon source needed by cells as they prepare for several rounds of division [9]. Moreover, activated T cells harvested near the peak of clonal expansion in immunized mice showed an increase in glucose accumulation [6]. We therefore decided to study whether this accumulation was due to increased rates of glucose uptake and whether or not it was correlated with adjuvant-mediated survival advantages.

Using 2-NBDG, a fluorescinated derivative of 2-deoxyglucose and known glucose-transport inhibitors, cytochalasin B and phloretin [10, 11], we measured glucose uptake and survival of T cells activated either in presence or absence of adjuvant effects induced by LPS. The results indicate that adjuvant-mediated survival effects do not require increased rates of uptake or intracellular accumulation of glucose.

8.2 Materials and Methods

8.2.1 T Cell Activation and Primary Cell Culture

Activated T cells were harvested from antigen treated mice as described elsewhere [6]. Briefly, Vβ3+ TCR bearing T cells were activated by injecting B10.BR mice via the tail vein with 0.1µg of the T cell superantigen Staphylococcal enterotoxin A (SEA; Toxin Technologies, Sarasota, FL) and 16 h later with 10 µg of bacterial lipopolysaccharide (LPS; from Salmonella typhosa; Sigma Aldrich, St. Louis, MO). Spleens were harvested 40 h after activation, red blood cells were lysed with ACK buffer (160 mM NH₄Cl, 10 mM KHCO₃,