

Dendritic cells mobilize NKT lymphocytes as adjuncts and adjuvants for anti-tumor immunity

Ralph M. Steinman

The Rockefeller University, New York, NY, USA

Abstract

Research on NKT cells has been greatly stimulated by the discovery that the synthetic glycolipid, alpha-galactosyl ceramide (α -Gal Cer) is presented on CD1d molecules to the invariant T cell receptor on mouse and human NKT cells. We find that the presentation of α -Gal Cer on dendritic cells (DCs) favors the mobilization of NKT cells that produce IFN- γ and exert CD1d-restricted cytolytic activity on tumor targets, thus providing an adjunct resistance role against tumors. Importantly, NKT cells can also serve as adjuvants for adaptive T cell immunity, by bringing about the full maturation of antigen capturing DCs *in vivo*. When mice are given a single dose of α -Gal Cer together with dying tumor cells, strong and specific CD4+ and CD8+ T cell immunity develops. We will discuss the latter findings, since they suggest ways to induce anti-tumor immunity without the need to genetically modify tumor cells with surrogate antigens or other means.

We have targeted irradiated, MHC negative, J558 plasmacytoma cells to DCs. According to the findings of Iyoda *et al.* (1) and Liu *et al.* (2), this is best achieved when the tumor cells are injected intravenously, whereupon a subset of CD8 α + spleen DCs selectively take up tumor cells or their fragments. In collaboration with Dr. Yang Liu, we find that the uptake of tumor cells is followed by antigen processing and presentation, as monitored with CD8+ TCR transgenic T cells specific for the classical P1A tumor antigen. To induce DC maturation, which is necessary to avoid the normal function of DCs in inducing peripheral tolerance, we have compared ligands for toll like receptors (polyIC and LPS), agonistic anti-CD40 monoclonal antibody, and NKT lymphocytes mobilized by the CD1d binding glycolipid, α -Gal Cer as described by Fujii *et al.* (3, 4). Mice injected simultaneously *i.v.* with tumor cells and glycolipid develop protective immunity. After one vaccination, immunity is long lived (>2 months), specific for MHC class I bearing J558 tumor cells and not other tumors from BALB/c mice, and requires both CD4+ and CD8+ T cells. TLR ligands and anti-CD40 antibody do not by themselves elicit protection, even though they do induce many surrogate markers of DC maturation. Protective immunity can also be generated to A20 lymphoma cells, but there is no cross protection observed to A20 in J558 immune mice, or vice versa. DCs from mice injected with irradiated tumor and α -Gal Cer transfer immunity to naive recipients. Therefore DCs which capture tumor cells *in vivo*, if they also mature in response to innate NKT lymphocytes, initiate strong adaptive tumor immunity.

References

1. Iyoda T, Shimoyama S, Liu K, Omatsu Y, Akiyama Y, Maeda Y, Takahara K, Steinman RM, Inaba K. The CD8+ dendritic cell subset

selectively endocytoses dying cells in culture and *in vivo*. *J Exp Med* 2002; **195**: 1289-1302. (PMID: 12021309)

2. Liu K, Iyoda T, Saternus M, Kimura Y, Inaba K, Steinman RM. Immune tolerance after delivery of dying cells to dendritic cells *in situ*. *J Exp Med* 2002; **196**: 1091-1097. (PMID: 12391020)
3. Fujii S, Shimizu K, Smith C, Bonifaz L, Steinman RM. Activation of natural killer T cells by alpha-galactosylceramide rapidly induces the full maturation of dendritic cells *in vivo* and thereby acts as an adjuvant for combined CD4 and CD8 T cell immunity to a coadministered protein. *J Exp Med* 2003; **198**: 267-279. (PMID: 12874260)
4. Fujii S, Liu K, Smith C, Bonito AJ, Steinman RM. The linkage of innate to adaptive immunity via maturing dendritic cells *in vivo* requires CD40 ligation in addition to antigen presentation and CD80/86 costimulation. *J Exp Med* 2004; **199**: 1607-1618. (PMID: 15197224)