[haematologica reports]
2006;2(3):35-36

Single cell monitoring of trogocytosis and killing in lytic synapses between anaplastic large cell lymphoma and activated human γδ T lymphocytes

Fournié JJ
Gertner J
Poupot M
Wiedemann A

Groupe d'Etude des Antigènes Non-Conventionnels, Departement Oncogénèse & Signalisation dans les Cellules Hématopoiétiques, Unité 563 de l'Institut National de la Santé et de la Recherche Médicale, Centre de Physiopathologie de Toulouse Purpan, Toulouse, France

Circulating human γδ T lymphocytes comprise a unique lymphoid cell subset exerting strong cytolytic activity against cancer cells.[1] In healthy adults, around 1% of peripheral blood mononuclear cells are γδ T lymphocytes, most of which harbour a $CD3^+$ $CD4^-$ $CD8^-$ $NKG2D^+$ phenotype and express the same TCR Vγ9/Vδ2 gene-encoded receptor for antigens. These lymphocytes often referred to as Vγ9/$Vδ2^+$ T cells do respond to both human cancer cells and tuberculosis infection, by the selective recognition of HLA-unrestricted antigens with non-peptide phosphoesters structures, the phosphoantigens.[2]

Natural phosphoantigens are thus produced by both mycobacteria and by human cancer cells, although the metabolic pathways to produce phosphoantigens differ in both types of producing cells. While mammalian cells and notably human tumours produce the phosphoantigens isopentenyl pyrophosphate and dimethylallylpyrophosphate through the mevalonate pathway for cholesterol biosynthesis, bacteria produce structurally related phosphoantigens like hydroxyldimethylallylpyrophosphate through a non-mevalonate pathway unique to the eukaryotic world.[3] Since therapeutic aminobisphosphonates like Aredia™ or Zometa™ target the farnesylpyrophosphate synthase enzyme from the mammalian mevalonate pathway, this drug induces bioaccumulation of endogenous phosphoantigens in treated cells.[4] Thus as recently discovered by Kunzmann and colleagues, Vγ9/$Vδ2^+$ T cells are strongly activated in cancer patients with multiple myeloma receiving Zometa™ or Aredia™ treatment for their bone-metastasis, and induced myeloma reductions.[5,6] Activating Vγ9/$Vδ2^+$ T cells *in vivo* in cancer patients either through Phosphostim™[7] or Zometa™[8,9] thus provides a novel means for anticancer immunotherapy. It is therefore of interest to identify other types of malignant diseases which may be targeted by Vγ9/$Vδ2^+$-T cell recognition and cytolytic activity.[10]

Like other cytotoxic lymphocytes, human γδ T cells expressing Vγ9/$Vδ2^+$-encoded TCR cells bind to antigen cells through immunological synapses,[11] acquire patches of their plasma membrane (trogocytosis) [12] and eventually kill these targets.[13] Although the process of trogocytosis mediated at the lytic synapse by cytolytic lymphocytes has recently been described,[14] there is of as yet no clear understanding of how the delivery of cytolytic granules to target cells is associated to their trogocytosis, nor of their temporal relationship.[15] Here using flow cytometry and time-lapse confocal video microscopy, we analyzed at the single cell level both features between human Vγ9/$Vδ2^+$ T lymphocytes preactivated with synthetic phosphoantigens or with zometa and anaplastic large cell lymphoma (ALCL) cell lines.[16] We found that both trogocytosis and perforin release were simultaneously initiated by formation of the immunological synapses. However, the target cell death stopped trogocytosis while the release of lytic granules at the synapse continued. Since nibbling of the target depended upon its viability, this demonstrated that the target cells controlled its trogocytosis together with the effector lymphocytes. In the reverse direction, the target cell also mediated a weak trogocytosis of its effector, in which case however the delivery of lytic granules from the ALCL cancer cells (contre-attaque) was unadjusted, and missed its objective. This first report for a dissociation of trogocytosis and cytotoxic response at the single cell level of lytic synapses (Figure 1) suggests that in this context, the purpose of trogocytosis could be to polarize the secretion of cytolytic granules.

Thus the strong reactivity of human Vγ9/Vδ2δ T lymphocytes to anaplastic large cell lymphoma triggers their specific lysis. These *in vitro* findings evidence the potential of activating γδ T cells for

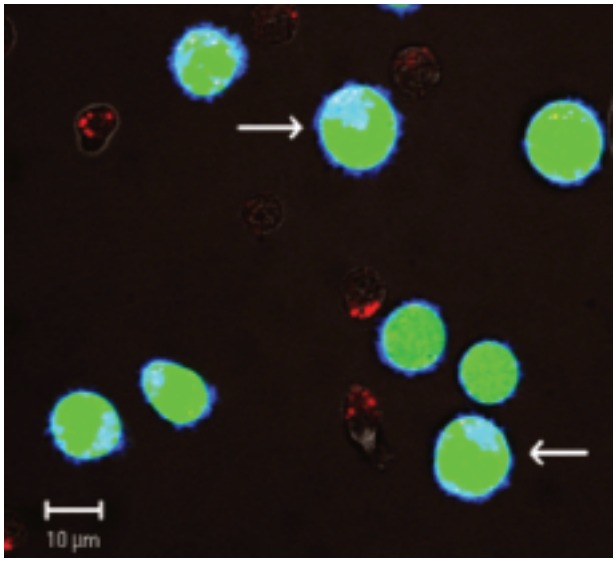

Figure 1. Lytic attack of ALCL tumour cell targets by human Vγ9/Vδ2⁺ T lymphocytes. Confocal microscopy showing γδ T cells with perforin granules (red) attacking the ALCL tumour cells (blue membrane, green cytoplasmic stain for viability).

immunotherapies of ALCL, a recently classified hematological malignancy and broadens their possible therapeutic use.

References

1. Kabelitz D, Wesch D, Pitters E, Zoller M. Potential of human gammadelta T lymphocytes for immunotherapy of cancer. Int J Cancer 2004;112:727.
2. Bonneville M, Fournie JJ. Sensing cell stress and transformation through Vγ9Vδ2 T cell-mediated recognition of the isoprenoid pathway metabolites. Microbes Infect 2005;7:503.
3. Fournie JJ, Bonneville M, Romagne F. Mechnanisms of action of non peptide antigens actiuvating human Vγ9Vδ2 T cells and their potential use for immunointervention. Current Medicinal Chemistry_Anti-Inflammatory and Anti-Allergy Agents 2005;4:161.
4. Gober HJ, Kistowska M, Angman L, Jeno P, Mori L, De Libero G. Human T cell receptor gammadelta cells recognize endogenous mevalonate metabolites in tumor cells. J Exp Med 2003;197:163.
5. Kunzmann V, Bauer E, Wilhelm M. γ/δ T-cell stimulation by pamidronate. N Engl J Med 1999;340:737.
6. Kunzmann V, Bauer E, Feurle J, Weissinger F, Tony HP, Wilhelm M. Stimulation of gammadelta T cells by aminobisphosphonates and induction of antiplasma cell activity in multiple myeloma. Blood 2000;96:384.
7. Bennouna JJ, Medioni F, Rolland J, Misset M, Campone H, Sicard J et al. Phase I clinical trial of Bromohydrin Pyrophosphate BrH-PP (Phosphostim) a Vγ9Vδ2 T lymphocytes agonistin combination with low-dose interleukin-2 in patients with solid tumors. (abstract # 2536). J Clin Oncol 2005;23:174s.
8. Dieli F, Gebbia N, Poccia F, Caccamo N, Montesano C, Fulfaro F, et al. Induction of gammadelta T-lymphocyte effector functions by bisphosphonate zoledronic acid in cancer patients *in vivo*. Blood 2003;102:2310.
9. Mariani S, Muraro M, Pantaleoni F, Fiore F, Nuschak B, S. Peola, et al. Effector gammadelta T cells and tumor cells as immune targets of zoledronic acid in multiple myeloma. Leukemia 2005; 19:664.
10. Blattman JN, Greenberg PD. Cancer immunotherapy: a treatment for the masses. Science 2004;305:200.
11. Favier B, Espinosa E, Tabiasco J, Dos Santos C, Bonneville M, Valitutti S, et al. Uncoupling between immunological synapse formation and functional outcome in human gamma delta T lymphocytes. J Immunol 2003;171:5027.
12. Espinosa E, Tabiasco J, Hudrisier D, Fournie JJ. Synaptic transfer by human gamma delta T cells stimulated with soluble or cellular antigens. J Immunol 2002;168:6336.
13. Sicard H, Al Saati T, Delsol G, Fournie JJ. Synthetic phosphoantigens enhance human Vγ9Vδ2 T lymphocytes killing of non-Hodgkin's B lymphoma. Mol Med 2001;7:711.
14. Poupot M, GertnerJ, Fournié JJ. Molecular transfers through transient lymphoid cell-cell channels. In Cell-Cell channels, Vol. 1. F. Baluska, D. Volkmann, and P. W. Barlow, eds. Landes Bioscience, p. in press.
15. Poupot M, Pont F, Fournie JJ. Profiling blood lymphocyte interactions with cancer cells uncovers the innate reactivity of human gamma delta T cells to anaplastic large cell lymphoma. J Immunol 2005;174:1717.
16. Gertner J, Poupot M, Wiedemann A, Fournié JJ. Trogocytosis and cytotoxicity at the single-cell level of lytic synapses between anaplastic large cell lymphoma and human gamma delta T cells. (submitted for publication).