Biserka Radošević - Stašić[1], Ines Mrakovčić - Šutić[2], Marija Šimin[3], Daniel Rukavina[4]

Protitumorska učinkovitost intrahepatičnih naravnih celic ubijalk T pri živalih

Antitumor Efficancy of Intrahepatic Natural Killer T Cells in Animal Models

IZVLEČEK

KLJUČNE BESEDE: ubijalke naravne, novotvorbe – imunologija

Naravne celice ubijalke T (NKT), ki izražajo invariantni T-celični receptor in označevalce celične vrste NK (NK1.1 in IL-2Rβ), so v zadnjih letih postale pomemben predmet raziskovanja, saj so očitno povezane z naravno odpornostjo za tumorje, nekatere povzročitelje okužb in za nastanek avtoimunosti. Kako te celice delujejo, še ni znano. Ve pa se že, da je njihovo delovanje omejeno s CD1, saj se vežejo z glikolipidnimi ligandi, proteini vročinskega šoka in dugimi dejavniki, povezanimi s stresom. Verjetno je, da so pomembne za spoznavo in prenos informacij, ki nastanejo ob nastanku tumorjev ali pa ob poškodbi telesnih celic. Posredno lahko sprožijo dejavnost imunskega sistema ali neposredno zavrejo tumorsko rast. Nadalje lahko izdelujejo različne citokine, ki vplivajo na ravnovesje med celicami TH1 in TH2.

Celice NKT zelo verjetno nastajajo tudi zunaj priželjca, najverjetneje v jetrih. V naši raziskavi smo ugotovili, da pri miših nastanek jetrnih celic NKT sprožijo:

a) delna hepatektomija (pHx),
b) vbrizganje streptozotocina (sredstvo, ki izzove nastanek avtoimunskega diabetesa) in
c) vbrizganje peptidoglycan monomera – PGM in PGM-Zn, ki ju pripravljamo iz po Gramu pozitivnih bakterij.

V navedenih poskusih smo ugotovili, da so pravkar osamljene MNLC iz jeter in vranice za celice YAC-1 in singenske timocite močno ubijalske, kar spodbuja domnevo, da so avtoreaktivne jetrne celice NKT zelo pomembne pri uravnavanju protitumorske imunosti in za razmnoževanje epitelijskih celic.

ABSTRACT

KEY WORDS: killer cells natural, neoplasms – immunology

Natural killer T (NKT) cells, which co-express the invariant T cell receptor and markers of NK cells lineage (NK1.1 and IL-2Rβ) have become a major focus in the studies of the innate immune response to tumors and infectious diseases, as well as in autoimmunity, but the precise immunological function of these »primitive« T lymphocytes is still not well defined. As

[1] Prof. dr. sc. Biserka Radošević - Stašić, Department of Physiology and Immunology, Faculty of Medicine, University of Rijeka, Ulica braće Branchetta 20, HR – 51000 Rijeka, Croatia.
[2] Dr. sc. Ines Mrakovčić - Šutić, Department of Physiology and Immunology, Faculty of Medicine, University of Rijeka, Ulica braće Branchetta 20, HR – 51000 Rijeka, Croatia.
[3] Marija Šimin, dr. med., Department of Physiology and Immunology, Faculty of Medicine, University of Rijeka, Ulica braće Branchetta 20, HR – 51000 Rijeka, Croatia.
[4] Prof. dr. Daniel Rukavina, Department of Physiology and Immunology, Faculty of Medicine, University of Rijeka, Ulica braće Branchetta 20, HR – 51000 Rijeka, Croatia.

CD1-restricted cells they interact with glycolipid ligands, heat shock proteins and other »stress« or »danger« signals produced by tumors or injured self cells, transferring important information to the rest of the immune system or directly suppressing the tumor growth. Furthermore, depending on the stimulus, they produce various immunomodulatory cytokines, affecting the TH1/TH2 immune balance.

Since particularly the liver might be a site of extrathymic generation of the NKT cells, we demonstrate herein evidence that in mice these cells might be induced by: a) partial hepatectomy (pHx), b) injections of streptozotocin (provoking autoimmune diabetes) and c) injections of peptidoglycan-monomer-PGM and PGM-Zn, prepared from Gram + bacteria. The data also showed that in all experimental models freshly isolated hepatic and particularly splenic MNLC became markedly cytotoxic to YAC-1 cells, as well as to syngeneic thymocytes, suggesting that autoreactive NKT cells in the liver might be key players in the regulation of anti-tumor immunity and epithelial cell proliferation.

INTRODUCTION

Cells of the innate immune system have not only the responsibility of surveying and »informing« the host of a breach in integrity, but also intrinsic antitumor effector functions, including lysis of tumor cells and the production of cytokines that inhibit tumor growth and block angiogenesis. NK, NKT and gamma delta T cells express activating receptors such as NKG2D that recognize MHC class I chain-related (MIC) or ULI6-binding proteins that become upregulated on tumor cells, while some NKT cells and gamma delta T cells also express T cell receptors with restricted diversity that recognize tumors through lipid or protein antigens presented in the context of MHC proteins *(1)*. Therefore, cells of innate immune system respond to »danger« signals, which can be provided by growing tumors as a consequence of the genotoxic stress of cell transformation and disruption of the surrounding microenvironment *(2)*. Under ideal conditions these signals will induce inflammation, activate innate effector cells with antitumor activity and stimulate professional antigen-presenting cells (APS) to engulf tumor-derived antigens and migrate to draining lymph nodes to trigger an adaptive response by T and B lymphocytes. However, this well-orchestrated surveillance operation is often ineffective, since progressing tumors exhibit various mechanisms that promote evasion from immune recognition *(3)*. The strategies which induce the proliferation of innate cells or APC or harnessing of innate immune cells as effectors therefore represent the current challenges in cancer immunotherapy *(1)*.

In this context, it was postulated that the adult liver of mice and humans might be a crucial organ in the first line of host defence against bacterial infections and hematogenous tumor metastases, since it contains a unique phenotypic distribution of Kupffer cells, NK cells and NKT cells responses. The later, NKT cells, are a highly conserved subset of primitive lymphocytes, which in phylogenetic sense stand between the NK and T cells and bear the surface receptors of both cell types *(4–7)*. Most of them constitutively express IL-2Rβ-chain (IL-2β) and have lower surface density of αβ T cell receptor (or CD3) (termed intermediate TRC; TCR^{int}) *(4–9)*. Their ontogeny is disputed, but $NK1.1^{+}$ $CD3^{int}CD4^{low}$ T cells might be generated by an alternative intrathymic pathway without passing through the double-positive $CD4^{+}8^{+}$ stage, as well as extrathymically in multiple sites in the body (liver, uterus, small intestine, salivary gland) *(6, 7)*. Both pathways are totally repressed in normal euthymic mice and became active only in the cases of severe T depletion. More importantly, these non-traditional T cells are characterized by restricted TCR repertoire, having the unusual property of being readily stimulated by non-protein components of microorganisms and some self-antigens, presented in the context with monomorphic MHC class I antigens, such as CD1 and TL in mice, and non-classical HLA class I antigens in humans (HLA-E, -F and -G) *(3–9)*. Recognizing