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Description of invariant NKT cells

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Abstract

Recently discovered subpopulations belonging to cell groups called “invariant” NKT, also referred to as type I NKT or classical NKT cells, appear to have a unique impact on the tumor microenvironment. Considering the characteristics of secreted cytokines, production of chemokines and the capability of cell cytotoxic activity, these cells have a significant function in being immune to bacteria, parasites, viruses, autoimmune and tumor diseases. The profile of secreted cytokine is probably decisive in reference to the distinct functions of the subpopulation, some of them exhibit immunosuppressive activity inhibiting an anticancer immune response, and the other stimulate the organisms for eradicating the tumor. Currently, it has been discovered six fundamental, functionally distinct subpopulations of iNKT cells (NKT1, NKT2, NKT10, NKT17, NKTreg, NKT_{FH}).

Key words: tumor microenvironment; invariant natural killer T cells; iNKT; tumor; immunology; cytokines

Introduction

A malignant tumor consists not only of tumor cells but also stroma, blood vessels and various cells of the immune system. These create a tumor microenvironment. The tumor development is influenced in diverse ways by tumor-infiltrating immune cells. On the one hand, some of these cells are able to kill tumor cells, e.g., cytotoxic T lymphocytes or natural killer cells (NK), on the other hand, some of them promote tumor development, e.g., MDSC or regulatory T cells (Treg). In connection with the immunosuppressive activity of certain cells on the immune system, the organism is not capable of detecting cancerous changes in cells and cannot respond to them. It might also be the case that the organism responds to these cells, however, this anticancer response is too weak to stamp out the disease. In order to eradicate the tumor more efficiently, the scientists seek to discern cells of this microenvironment, to identify mechanisms of these cells and inhibit these which are responsible for "immune escape". In the microenvironment, mutual interactions between cancer cells and cells of the immune system are observed, in a direct or indirect way through the secretion of various immunostimulatory or immunosuppressive factors [1, 2, 3].

Characteristics of iNKT cells

The extensive studies conducted in recent years indicate that "invariant" NKT cells (iNKT), also called type I NKT cells, constitute likewise crucial elements of the microenvironment [4, 5]. They are the main NKT cell subpopulation and demonstrate, inter alia, the expression of invariant TCR Vα24-Jα18 receptors in humans combined with a heterogeneous Vβ11 [6]. At the beginning, NKT cells were defined as a combination of T lymphocytes and NK cells. They expressed, respectively, CD3, TCR αβ, CD56, CD161, NKG2D and CD94. Nowadays, this definition is not up to date anymore as NKT cells appear to be a new and separate subpopulation and they constitute a broader family of cells [7]. NKT cells differ from the conventional T lymphocytes, which recognize peptide antigens, since they detect lipid and glycolipid antigens using TCR receptors. Type I NKT cells are able to recognize the following antigens: αGalactosylceramide (α-GalCer), endogenic lipids and exogenic bacterial ligands [7, 8, 9]. A syntetic glicolipid α-GalCer is applied commonly, and it is the most recognizable exogenous lipid antigen used for the activation of type I NKT cells. It was isolated from the marine sponge *Agelas mauritanus* and its symbiotic microorganisms [7, 10]. Antigens are presented to NKT cells via CD1d nonpolymorphic molecules located chiefly on B lymphocytes, dendritic cells and macrophages – cells belonging to the major histocompatibility complex (MHC) [6, 7, 8, 9]. iNKT cells are activated directly, whereas TCR interferes with a ligand presented by a CD1d protein on professional antigen-presenting cells (APC). For instance, dendric cells (DC) present glycosphingolipid (such as, synthetic α-GalCer) and stimulate in this way iNKT cells to the production of cytokines, e.g., IFN-γ and IL-4 [7]. However, cytokines secreted from activated cells presenting an APC antigen, e.g., IL-12 or IL-18, are also able to activate NKT cells indirectly and independently from CD1d [7, 11].

Mature iNKT cells may be classified into three phenotypically distinct subpopulations: CD4+CD8-, CD4-CD8+ or CD4-CD8- [7, 12]. The subset of CD8+ cells is small and is present solely in humans [7]. Furthermore, mature iNKT cells show a phenotype characteristic of activated and memory cells (CD69+,CD44+, CD122+, CD62L-) [13].

Classical NKT cells are located in thymus, lymph nodes, bone marrow, peripheral blood, spleen or lungs [14]. The percentage of this cells constitutes a little subgroup which fluctuates between 0.01-0.5 % of all T lymphocytes in the mouse [7]. The biggest number of iNKT cells was observed in the mouse's liver, approx. 10-30 % of all T lymphocytes. In the human liver, on the other hand, this percentage is 10 times lower [7, 15]. The percentage for human spleen, blood, bone marrow and lymph nodes are similar. Additionally, a rather numerous population was noted in the white adipose tissue. Although the population of iNKT cells is not big, they are capable of producing cytokines after being activated. This may have a various impact on the immune system [7].

NKT cells have particular phenotypical and functional qualities which combine qualities of both the innate and acquired immune response. They function as an element of non-specific immunity thanks to their sudden response to an antigen. iNKT cells appear in the place of an infection or inflammation as one of the first cells [7, 16]. The qualities of NKT cells are influenced by both the way of their activation and their localization in the organism. They indicate a proinflammatory and anti-inflammatory activity [17]. Extensive studies are conducted on this small but extremely important cell subpopulations as their various influence on the immune system has not been profoundly examined. It is regarded that iNKT cells are crucial due to their resistance to viruses, bacteria, parasites, autoimmune diseases and tumors [11, 18]. The row of proinflammatory typical of Th2 cells (i.a., IL-4 and IL-10) cytokines and anti-inflammatory typical of Th1 cells (i.a., IFN-γ i TNF-α) cytokines is secreted by activated iNKT cells. The proinflammatory cytokines take part in humoral immune responses, and anti-inflammatory cytokines are characteristic for cellural immune response [9, 14]. iNKT cells can produce cytokines in a fast and sudden way and chemokines. Moreover, these cells are capable of cytotoxic activity. They serve dual function in the immune system: as regulatory and effector cells [5, 9, 15]. Various cytokines have different tasks, some of them exhibit immunosuppressive activity inhibiting an anticancer immune response, and the other stimulate the organisms for eradicating the tumor [12, 14]. In addition to the production of IFN-γ and IL-4, these cells are able to produce a row of different cytokines, such as, IL-21, IL-17, IL-10, IL-2, TNF-α, TGF-β and GM-CSF [5, 13], moreover, they can produce chemokines, such as eotaxin, RANTES, MIP-1α or MIP-1β [5]. It should be noted that the fast synthesis of IL-4 and IFNγ by iNKT cells after adding α-GalCer is a defining characteristic of iNKT cells. A constantly high level of mRNA both for IL-4 and IFN-γ enables such effective cytokine production. Moreover, transcription factors regulating the gene expression for cytokines in classical T lymphocytes (GATA-3, RORγt or T-bet) have an important function in relation to iNKT cells. However, mechanisms of iNKT cell activity remain less explored than in T lymphocytes. At the same time, iNKT cells may show T-bet as well as GATA-3 expression leading to mRNA transcription for IFN-γ and IL-4. This is an opposition to conventional T lymphocytes in which T-bet suppresses GATA-3 expression and GATA-3 inhibits T-bet expression. Considering that, further studies are needed in order to comprehend transcription regulation of producing cytokines by iNKT cells [13].

Additionally, iNKT cells are able to destroy directly tumor cells after the stimulation as a result of cytolysis induced by perforins, Fas-FasL axis, TRAIL and granzyme B [7, 13]. It has been observed that the expression of a CD1d molecule within tumor cells increases their vulnerability to lysis exerted in vitro by NKT cells [19, 20].

Therefore, it was hypothesized that there exists a correlation between reduced metastatic ability and increased CD1d expression in tumor cells [20]. However, the main function in immune control is served by mobilization and activation of other cytolytic cells through classical NKT cells producing Th1 cytokines. The studies suggest that various subsets of NKT cells, which express different surface markers or distinct transcription factors, may serve miscellaneous functions [7]. It is suggested that iNKT cells should not be treated as a homogenic population. The nature of these cells may be more complex than it was expected [11].

Through the release of cytokines, NKT influence functions of many immune system cells, e.g., B lymphocytes, T lymphocytes CD4+/CD 8+, macrophages, neutrophils, dendritic cells and NK cells. Activation of NK cells is crucial for an effective anticancer response. Recent studies have showed that iNKT cells recognize bacterial antigens, react on them and take part in destroying them. Activating NK cells as a result of generation IFN-γ is an important function of iNKT cells at early infection stage [13]. Recent studies have widened the range of cells influenced by iNKT cells. Interactions among iNKT cells, T-regulatory lymphocytes, MDSC, M2 macrophages and Tγδ lymphocytes were described in recent researches. Furthermore, it was concluded that NKT cells may inhibit an anticancer immune response [9]. The decrease of anti-cancer immunity occurs through the interaction of iNKT cells with immunosuppressive cells, including myeloid-derived suppressor cell (MDSCs) [7, 21]. iNKT cells which are activated, qualitatively and quantitively modulate the Treg function, whereas Treg may suppress functions of iNKT cells. A similar regulation was observed between cells iNKT and type II NKT. Type II NKT appear to suppress the activation of iNKT [13].

Several new subpopulations of iNKT cells have been recently discovered. They differ from each other in phenotypical and functional terms [11, 14, 22]. They develop naturally in thymus [7] and their maturation is conditioned by the expression of signal-transduction molecules (i.e., SAP/Fyn) and by transcription factors (i.e., Egr2, PLZF, RORγt, T-bet, GATA3). On the basis of released cytokines and kinds of transcription factors, the following subpopulations of iNKT cells were differentiated among iNKT: NKT1, NKT2, NKT17, NKT_{FH}, NKT10 [11, 22] and also recently NKT-reg (FoxP3 +) [14].

Conclusions

It appears that establishing the relevance of this minor subpopulation in biology and pathogenesis of a given disease is possible exclusively by differentiating all currently known populations of iNKT cells and their detailed examination [11, 14]. Depending on the type of analyzed tumor, functional and quantitative disorders among this cell group were observed [12]. Furthermore, the increase of percentage of iNKT CD4+ cells in reference to iNKT CD4- cells in the tumor microenvironment was proved. It is possible that this promotes the development of the disease or its recurrences in connection with ineffective anticancer protection. iNKT CD4+ cells release chiefly Th2-type cytokines suppressing the expansion of CD8+ T lymphocytes [23]. Every subpopulation of iNKT cells may have a unique influence on the tumor microenvironment due to the characteristics of released cytokines. Moreover, iNKT cells constitute alternative in the immunotherapy of tumors in relation to classical T lymphocytes.

This analysis may provide more detailed information on the tumor microenvironment and may help with differentiating immunosuppressive and immunostimulant cells. The modulation of iNKT cell activity may serve as a useful tool in strategies of anticancer and anti-inflammatory immunotherapy. In the future, such a functional differentiation may influence blocking cells which inhibit the immune system and stimulation of anti-cancer cells. This opens up the new possibility for patients resistant to the standard treatment or patients with recurrent disease [24].

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