Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

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Letter to the Editor

Can we define $CD3^+CD56^+$ cells as NKT cells with impunity?

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To the Editor,

Zingaropoli M. A. et al. [22] on 13 November 2020 published a paper showing a significant reduction of NKT cells in patients with severe COVID-19 pneumonia. The Authors used multiparameter flow cytometry to analyze a broad range of peripheral blood leucocytes and their subsets, including T cells, NK cells, and NKT cells, in blood samples from COVID-19 subjects and healthy donors. As a result, NKT cell reduction was associated with the severity of the disease. Although this report is very interesting, there are also some points of our concern. In the paper, the Authors defined NKT cells based on co-expression of CD3 and CD56 molecules, and the median percentage of NKT cells in COVID-19 patients was 3.8% (IQR 2.4–7.0), and 8.8% (IQR 5.8–12.1) in healthy donors. Nonetheless, both the way of identification of NKT cells, as well as their frequency established in the paper, are, in our opinion, incorrect.

NKT cells gained their name in reference to the NK cells, as they co-express T-cell receptor (TCR), together with surface receptors characteristic for NK cells [14]. However, it is now widely known that NKT cells are a small population of thymus-derived T cells, restricted by non-classical MHC class I molecule CD1d. NKT cells express an evolutionary conserved TCR with an invariant α-chain, Vα24-Jα18 in humans, paired with Vβ11 [19]. This invariant TCR combination gives NKT cells the specificity for glycolipid antigens, presented by CD1d molecule. Thus these cells should be called iNKT cells [5].

iNKT cells can be detected by the standard flow cytometry method. The most specific tool for iNKT cell identification in both mice and humans are αGalCer/CD1d tetramers [2,17,20]. The ability iNKT cells to selectively bind CD1d molecules loaded with α-GalCer has also been used to develop MHC-peptide monomers bound to polymers of glucose, called dextramers. Dextramer reagents carry more MHC molecules; thus, they produce a stronger signal than conventional MHC multimers [1]. Alternatively, iNKT cells can be detected using a recently generated mAb 6B11 against the conserved CDR3 region of the canonical Va24-Ja18 TCR [6,8].

In our paper [15] we compared different iNKT cell detection methods, including anti-CD3 and anti-CD56 monoclonal antibodies (mAbs), 6B11 mAb, against the conserved CDR3 region of the canonical Vα24-Jα18 TCR, and CD1d multimers loaded with α-GalCer. Our results show that similar results, in terms of iNKT cell counts in peripheral blood, are obtained with 6B11 mAb and α-GalCer-loaded CD1d dextramers (app. 0.3–0.5% of CD3 cells), while $CD3^+CD56^+$ cells constitute ~13% of T cells. Our results are consistent with other reports on the iNKT and $CD56^+CD3^+$ T cell counts in human blood. As an example, the mean ratios of 6B11-iNKT cells and Valpha24-Vbeta11 iNKT cells among T lymphocytes were 0.54% and 0.31%, respectively [9], while the average iNKT cell counts in Caucasian children and adolescents are in a range of 0.003–0.775% of peripheral blood T cells [3]. Bojarska-Junak et al. reported that the percentage of iNKT cells is much lower than the percentage of $CD3^+CD56^+$ lymphocytes in healthy individuals (iNKT cells constitute ~7.5% of T lymphocytes) [4]. Similar results were obtained by our group (iNKT cells constitute ~13% of T lymphocytes).

It is also worth mentioning that although the Authors have thoroughly discussed their observations of a lower percentage of NKT cells in COVID-19 patients with a number of publications, all of the cited studies applied standard identification of iNKT cells using αGalCer/CD1d tetramers [7,11–13,16,18,21].

Summarizing, we believe that the depletion in NK-like T cells observed in patients with COVID-19 by Zingaropoli M. A. et al., should not be treated as depletion in canonical NKT cell counts. Re-evaluation of this cell population using the method described by Godfrey et al. should be considered [10].

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