Applications in CAR T-cell Therapy: Dissecting Cellular Composition Using Single Cell Multiomics

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EDITORIAL COMMENTARY

Gr1 makes an unexpected cameo appearance in eosinophils

See corresponding article on Page 367

Eosinophils are granulocytes that serve an enigmatic function in the innate immune system, and develop in the bone marrow from hematopoietic progenitor cells. These granulocytic cells possess a broad range of immunomodulatory mediators including cytokines and chemokines that may be preformed and stored in their crystalloid granules, or newly synthesized upon receptor stimulation. Numerous receptors for many of these mediators are also present in eosinophils, including IL5 receptor and CCR3, the latter of which binds to several chemokines like eotaxin-1. Based on flow cytometry analysis of blood samples from mouse models, eosinophils have been assumed to express very little or no Ly6G, and Abs to this Ag have been used primarily as universal markers for neutrophils. Ly6G is a GPI-linked leukocyte surface Ag expressed in mouse neutrophils and is 1 of the 2 components of Gr1 (the other being Ly6C). Structurally, Ly6G is a member of a conserved family of leukocyte antigens that share LU domains (3-fold repeat Ly6 Ag/urokinase-type plasminogen activator receptor, uPAR). Like all GPI-linked antigens, Ly6G lacks a C-terminus domain extending into the cell cytoplasm to initiate binding to intracellular signaling pathways, so is unable to directly signal into the cell, although GPI-linked antigens may signal via lipid rafts. In mouse models that examine neutrophil function, Abs to Ly6G/Gr1 (clone 1A8 for Ly6G, clone RB6-8C5 for Gr1) have frequently been used in imaging blood and tissue neutrophils, and as tools for Ab-mediated depletion of neutrophils and subsets of monocytes from the blood. In many of these models, mouse eosinophils are typically described as Gr1− or Gr1lo.

Recently, in marked contrast to previous reports, it was found that Ly6G is readily detectable on mouse eosinophils, and that this surface Ag increases in response to elevated concentrations of IL5 in vitro or in vivo (Figure 1). While blood from wild type Balb/c mice exhibited relatively few Gr1+ eosinophils, sensitization and challenge of mice with Aspergillus fumigatus increased Gr1+ eosinophils in the bone marrow but not spleen or lungs. Likewise, eosinophils from IL5 transgenic mice and bone marrow-derived eosinophils (bmEos) cultured in the presence of elevated IL5 concentrations showed increased Gr1+ eosinophils. These findings suggest that IL5 either directly or indirectly induces Gr1 expression in eosinophils. Detection of Gr1 was confirmed with another specific antibody to one component of Gr1 (Ly6G), since the Gr1 Ab may cross react with Ly6C. These findings corroborate with numerous reports indicating that wild type eosinophils are positive for Gr1 in specific tissue microenvironments in spleen and lungs.

There was also a mouse strain difference in Ly6G expression in eosinophils. While Balb/c eosinophils predominantly express Ly6G, eosinophils from C57Bl/6 mice preferentially express Ly6C. This could account for the assumption that eosinophils are typically Gr1− or Gr1lo in earlier reports, depending on the Ab used. It is not clear if incubation of eosinophils from C57Bl/6 mice with IL5 will ultimately lead to a transition to Ly6G/Gr1.

When eosinophils were cultured from bone marrow, IL5 induced the development of Gr1+ and Gr1− eosinophils, while Gr1− eosinophils expressed higher levels of transcripts for the eosinophil granule proteins major basic protein-1 (MBP-1) and eosinophil peroxidase (EPX). These transcripts are usually detected at the promyelocytic stage of eosinophil development. When sorted, Gr1− eosinophils rapidly converted to the Gr1+ state in the presence of IL5, while Gr1+ cells maintained their profile. These observations suggest that Gr1− eosinophils may be less differentiated than Gr1+ cells, and that they have the capacity to up-regulate Gr1 and develop a more mature phenotype in the presence of IL5.

An interesting and important protocol development was described in this report in which the concentration of IL5 used to differentiate bmEos in culture could be reduced from 10 ng/ml to 5% of its original level. Cells cultured with 0.5 ng/ml IL5 retained functional and morphological similarity to those produced in the presence of 10 ng/ml IL5. Reducing the IL5 dosage to lower levels led to diminished generation of Gr1+ eosinophils in culture, thus further supporting the evidence that Gr1+ expression is regulated by IL5.

Understanding the function of Ly6G/Gr1 has remained a daunting challenge for leukocyte biologists. While anti-Ly6G prevents neutrophil migration in vitro in a mechanism involving direct association between Ly6G and β2 integrins, disruption of the Ly6G locus in the recently described Catchup mouse exhibited normal neutrophil functions in vivo in response to sterile and infectious stimuli. In eosinophils, chemotactic responses to eotaxin-1 in Gr1+ eosinophils were blunted relative to Gr1− eosinophils, despite a higher proportion of CCR3+ cells among the Gr1+ population. Further, addition of eotaxin-1 together with IL5 limited the expression of the Ly6G/Gr1 antigen.

Taken together, these findings suggest that, from a technical standpoint, investigators that plan to deplete neutrophils using anti-Gr1 or anti-Ly6G in mouse models will need to consider effects on Gr1+ eosinophils, particularly in models exploring IL5-dependent or Th2-associated mechanisms. Second, investigators will need to be vigilant in immunolabeling of Gr1+ cells in blood or spleen samples, as well as tissue sections. Third, this study raises important questions about the possibility that endogenous ligands for Ly6G may exert effects on eotaxin-1-induced chemotaxis.
More broadly, a careful analysis of eosinophils and IL5-mediated responses may reveal clues toward identifying a function for Ly6G. Future studies could examine the introduction of the IL5 transgene into Catchup mice and determine if Ly6G/Gr1 expression in eosinophils is modified in vivo, particularly in bone marrow. From this report, the observation that Gr1⁺ eosinophils exhibited reduced chemotactic responses to eotaxin-1 suggests an inhibitory role for Ly6G in chemotaxis. Since Ly6G is a GPI-linked surface antigen, it is not expected to bind to signaling molecules upon engagement of ligands or stimulation by other receptors such as the IL5 receptor. Mechanisms underlying the comparatively reduced chemotaxis in Gr1⁺ eosinophils are speculative at this time, but it could be predicted that Ly6G may interact with other extracellular molecules such as CCR3 to down-regulate chemotaxis (Fig. 1).

These findings could theoretically be applied to humans, as the human eosinophil expresses the related molecule uPAR, which belongs to the Ly6/uPAR family. Although mouse Ly6G and human uPAR may have different functions, understanding the function of Ly6G in the mouse warrants further investigation as a potential target for intervention. Future investigations are anticipated that will closely examine the function of Ly6G/Gr1 in eosinophils to resolve whether this surface antigen regulates chemotactic responses, as well as the identity of its endogenous ligand.

**REFERENCES**


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