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Technical note

T cell acquisition of APC membrane can impact interpretation of adoptive transfer experiments using CD45 congenic mouse strains

Kathy S. Cho, Ann B. Hill *

Oregon Health and Science University, Molecular Microbiology and Immunology, Portland, OR 97239, USA

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Abstract

Congenic mouse strains bearing allelic variants of CD45 are often used in adoptive transfer experiments. Here, we report that immune $CD8^+$ splenocytes of donor origin acquired the recipient's congenic CD45 marker during interaction with antigen-bearing cells, presumably as a result of membrane transfer upon dissolution of the immunological synapse. Acquisition of recipient marker by donor cells was most prominent after in vitro incubation with peptide for intracellular cytokine staining, where most of the antigen-bearing splenocytes are of recipient origin. In consequence, when antibodies against the recipient's congenic marker were used to distinguish donor and recipient populations, donor origin cells were incorrectly interpreted as being of recipient origin. This phenomenon may cause problems for interpretation of data in adoptive transfer experiments primarily when (a) staining for the recipient's congenic marker and (b) identifying antigen-specific populations by staining for intracellular cytokine.

Keywords: Membrane transfer; Adoptive transfer; CD45 congenic; Murine cytomegalovirus

1 . Introduction

Identifying and isolating cellular components of the adaptive immune response are important steps for understanding the complex interplay among various lymphocyte subsets during an immune response. A common technique for this purpose is the use of adoptive transfer experiments between congenic strains of mice. Congenic markers, such as CD45.1 and CD45.2, can then be used to distinguish donor and recipient cells. Most commonly, the experimental goal requires tracking the donor cell population. Sometimes, however, the impact of the transferred cells on the recipient's immune response is of interest, and this necessitates accurate identification of the recipient cell populations. We were interested in understanding the impact of transferred memory CD8 T cells on the primary immune response to murine cytomegalovirus (MCMV). We identified antigen-specific CD8 T cells by intracellular cytokine staining (ICS), and attempted to identify the response of the naïve recipient cells by staining for the recipient's CD45 congenic marker. During these experiments, we realized that donor cells had come to express the recipient's CD45 congenic marker, presumably due to acquisition of recipient cell membrane after antigen recognition. This caused a serious misinterpretation of the data when we used the recipient's congenic marker in attempt to identify the recipient T cell population.

* Corresponding author. Tel.: +1 503 494 0763.
E-mail address: hillan@ohsu.edu (A.B. Hill).

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2. Materials and methods

2.1. Mouse and virus strain

Mice congenic in CD45 at the Ly5 locus (C57BL/6J-Ly5.2 (CD45.2) and B.6SJL-Ptprca Pepcb/BoyJ-Ly5.1 (CD45.1)) were purchased from The Jackson Laboratory. Mice, aged −21–23 weeks, were intraperitoneally infected with 2×10^6 pfu MCMV strain MW97.01 which was derived from a bacterial artificial chromosome of Smith strain MCMV (Wagner et al., 1999). These mice were latently infected for approximately 6–16 weeks and were used as a source of transferred splenocytes. Uninfected congenic recipient mice were aged 12–18 weeks. Mice were housed at Oregon Health and Science University and all studies were approved by the Institutional Biosafety Committee and the Institutional Animal Care and Use Committee.

2.2. Surface and intracellular cytokine staining

Approximately 10^6 splenocytes were surface stained with the following antibodies: anti-CD8α Pacific Blue (53–6.7, eBioscience) at 1/300 dilution, anti-CD45.1 FITC (A20) at 1/300 dilution, anti-CD45.1 PE and PE-Cy7 (A20, eBioscience) both at 1/100 dilution, anti-CD45.2 PE (clone 104, eBioscience) at 1/100 dilution, anti-CD45.2 APC-Cy7 (clone 104, eBioscience) at 1:50 dilution and anti-CD45.2 APC (clone 104, eBioscience) at 1/300 dilution. The anti-CD45.1 antibody was isolated from hybridoma culture supernatant and conjugated to FITC (Sigma-Aldrich) using a standard conjugation protocol with cross-linker, SMCC (succinimidyl 4-(*N*-maleimidomethyl) cyclohexane-1-carboxylate, Pierce). M38-K^b tetramer was used at 1/600 dilution and was generated from the NIH Tetramer Facility. Staining with M38 tetramer was done for 1 h on ice. Staining for CD8α, CD45.1 and CD45.2 was performed overnight at 4 °C.

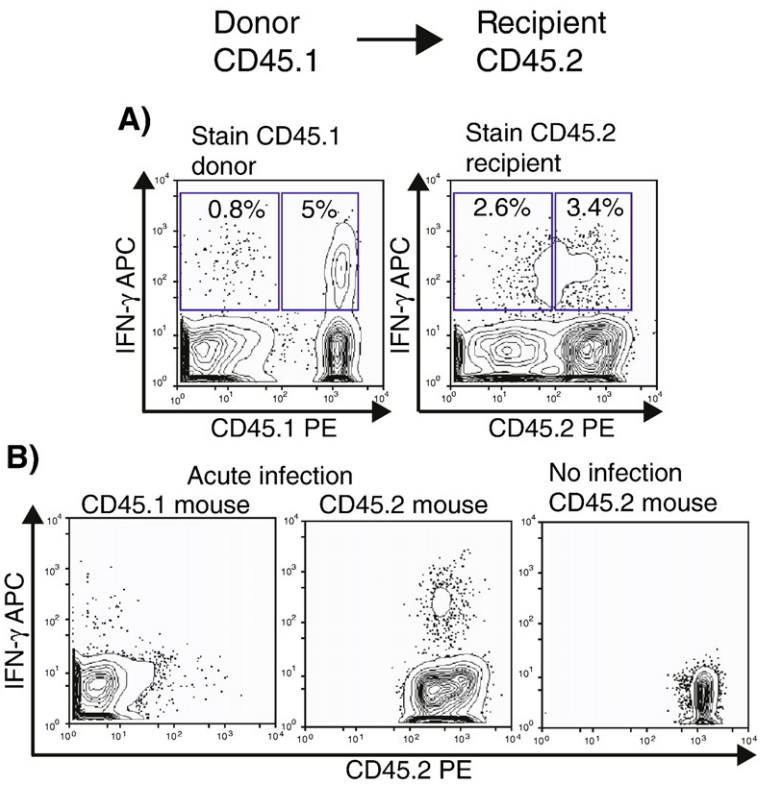

Fig. 1. Disparate determination of recipient response when staining for donor versus recipient congenic marker. Splenocytes from a latently MCMV-infected CD45.1 mouse were transferred to a naïve CD45.2 recipient, which was inoculated with MCMV the next day. One week later, splenocytes were stimulated with M38 peptide followed by ICS for IFN-γ and staining for CD8 and CD45.1 or CD45.2. FACS plots shown are from an initial $CD8\alpha^+$ gate. A) Comparison of staining for donor CD45.1 marker (left panel) or recipient CD45.2 marker (right panel). B) CD8 T cell response from infected CD45.1 and CD45.2 mice without adoptive transfer and naïve CD45.2 control mouse.