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Pregnancy-induced maternal regulatory T cells, bona fide memory or maintenance by antigenic reminder from fetal cell microchimerism?

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Long-term maintenance of immune components with defined specificity, without antigen is the hallmark feature of immunological memory. However, there are fundamental differences in how memory CD8⁺ compared with CD4⁺ T cells are maintained. After complete antigen elimination, CD8⁺ T cells can persist as a self-renewing numerically stable cell population, and therefore satisfy the most stringent definition of "memory." Comparatively, CD4⁺ T cell maintenance is considerably less stable, often requiring low-level antigen persistence or antigenic reminders. Recent studies show these basic memory features, classically ascribed to effector CD8⁺ and CD4⁺ T cells, extend to immune suppressive Foxp3⁺ regulatory CD4⁺ T cells (Tregs). In particular, gestational expansion and postpartum retention of maternal Tregs with fetal specificity may explain the protective benefits of primary pregnancy on complications in subsequent pregnancy. Herein, the possibility of ongoing antigenic reminders from fetal cell microchimerism in postpartum maintenance of maternal Tregs with fetal specificity is considered.

Keywords: T cells, immunological memory, regulatory T cells, pregnancy, microchimerism

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The mammalian immune system is endowed not only with efficient self, non-self discrimination, but also the ability to "remember" antigenic encounters. For immunologically foreign antigens, prior stimulation has the potential to prime long-term retention of "memory" immune cells with specificity to the inciting antigen. In turn, establishing the molecular and cellular requirements for immunological memory has critical implications for developing more durable vaccines and other immune modulatory therapies.

Emerging studies highlight an interesting discordance in necessity for antigen persistence in maintaining long-term retention of CD8⁺ compared with CD4⁺ T cells with defined specificity.[1-7] This is best illustrated by the dynamics of pathogen-specific CD8⁺ and CD4⁺ T cells after infection with viruses or other intracellular pathogens that do not cause persistence. While both T cell subsets expand robustly during acute infection, a numerically stable self-renewing pool of pathogen-specific CD8⁺ T cells is maintained indefinitely despite complete antigen elimination. By contrast, CD4⁺ T cells responding to the same acute infection undergo protracted, but stable contraction with an estimated half-life of 15 to 40 d.[8-10] This discordance may reflect the necessity for each T cell subset in host defense. For acute infection with viruses or other intra-cytoplasmic pathogens (e.g., influenza A, lymphocytic choriomeningitis virus, or *Listeria monocytogenes*) where protection is conferred by CD8⁺ T cells, these cells are chosen for selective retention. Comparatively for pathogens that primarily cause persistent infection and reside within the phagocytic vacuole of infected cells thereby escaping detection or elimination by CD8⁺ T cells (e.g., *Mycobacterium tuberculosis*, *Leishmania major*, or *Salmonella* spp.), pathogen-specific CD4⁺ T cells play a more dominant protective role.[11-13] Importantly however, while CD8⁺ T cell mediated protection against secondary

infection is maintained well after antigen elimination, protection by retained memory CD4+ T cells requires low-level antigen persistence. Accordingly for pathogens capable of establishing persistent infection, antigen elimination that occurs naturally or with adjunctive antimicrobials, accelerates contraction of pathogen-specific CD4+ T cells and overrides the protective benefits of prior infection.[14-16] Therefore, unlike CD8+ T cells, the long-term maintenance of CD4+ T cell memory appear to require more frequent, if not constant, antigenic reminders.

While the memory features of CD4+ T cells has been best characterized for IFN-γ producing Th1 cells, other CD4+ effector lineages (e.g., Th2 or Th17 cells) appear to share a similar potential for long-term retention.[7,10] By redirecting tools for tracking antigen-specific T cells, we and others have recently shown these memory features classically described for effector T cells also extend to immune suppressive regulatory CD4+ T cells (Tregs) identified by Foxp3 expression. Treg memory was first shown using transgenic mice where the model antigen, ovalbumin, could be inducibly expressed within the skin.[17] Primary dermal stimulation with this surrogate self-antigen primed expansion and retention of ovalbumin specific Tregs that dampens the severity of localized autoimmune reactions when this antigen was re-expressed ~30 d later. Likewise, a complementary study tracking Tregs after influenza A infection showed accelerated accumulation of virus-specific Tregs after secondary, compared with primary infection, which may be important for limiting pathological airway inflammation from over-exuberant effector CD8+ T cell activation.[18] Our own studies investigating maternal Tregs with specificity to the immune-dominant I-A^b:2W1$S_{55–68}$ peptide expressed as a surrogate fetal antigen during allogeneic pregnancy, showed Foxp3+ CD4+ T cells with this specificity progressively expand throughout gestation.[19] Interestingly after delivery of the fetus and other gross products of conception, maternal Tregs with fetal specificity were maintained at markedly enriched levels; and these cells re-expand with accelerated tempo during secondary pregnancy upon encounter with the same paternal-fetal antigen. Considering the necessity for expanded maternal Tregs in maintaining fetal tolerance during pregnancy,[19-24] these findings likely provide critical mechanistic insights for how primary pregnancy protects against complications stemming from fractured fetal tolerance in subsequent pregnancy.[19,20,25,26] In turn, applied to the basic biology of CD4+ T cells, these findings together establish Foxp3+ Tregs, like effector T cells, can persist as memory immune cells.

Given the discordance in necessity for antigen persistence in sustaining long-term retention of CD8+ compared with CD4+ effector T cells with defined specificity, these findings also open up exciting new questions regarding whether retained Tregs reflects bona fide memory or maintenance in response to antigen persistence. In the case of Tregs with specificity for surrogate-self ovalbumin antigen within the skin, ongoing stimulation is unlikely since naive ovalbumin specific T cells failed to proliferate after adoptive transfer without induced antigen expression.[17] Similarly, Tregs retained after influenza A infection are unlikely to reflect stimulation from residual viral antigen, since this pathogen is not known to cause persistent infection.[18] However in each of these models, the longer-term durability of Tregs, with specificity to either self or pathogen, remain undefined since the impacts of secondary antigen challenge were reported at most ~35 d after silencing primary antigen stimulation.[17,18] In our studies tracking maternal Tregs with surrogate fetal-2W1S specificity, enriched cells were maintained through at least 100 d postpartum despite progressively diminishing cell numbers.[19] In particular, the postpartum decay kinetics of maternal Tregs with fetal specificity (estimated $t_{1/2}$ of 25 d) show striking similarity with effector CD4+ T cells primed by acute infection.

On the other hand and in sharp contrast to the tempo of antigen stimulation that occurs after acute infection conditions, retained maternal Tregs with fetal specificity are likely to have more frequent antigenic encounters from fetal cells that establish microchimerism, analogous to low-level antigen stimulation in the later stages of persistent infection. Fetal cell microchimerism initiated during pregnancy and sustained postpartum probably occurs ubiquitously, but this phenomenon has become only recently widely appreciated with the use of molecular tools that allow these rare (~1 in 10^6) cells to be consistently identified.[27-29] Accordingly, antigenic reminder from fetal cell microchimerism may be pivotal for sustaining memory among pregnancy-induced maternal Tregs. Moreover, if maternal CD4+ Treg memory is sustained by fetal cell microchimerism, it would be interesting to consider the necessity for comparable antigenic reminders in maintaining regulatory CD8+ T cells shown in other contexts.[30-32] Along with the long-term maintenance of maternal cell microchimerism sustained by fetal Tregs in offspring,[33] this emerging body of evidence highlight remarkably potent and long-lived immunological programming that occurs naturally with the bi-directional transfer of cells and antigens between mother and fetus through in utero exposure.

Based on these findings, we propose important next steps are to more meticulously dissect the physiological milieu of pregnancy and in utero development that primes immunological tolerance and Treg memory. Taking cues from effector CD4+ T cell memory,[1-7,34] this will likely include interrelated contributions from naive cell precursor frequency, primary expansion magnitude, antigen avidity, and response to cytokine growth factors, along with increased frequency of antigenic reminders. Furthermore, given the potential for Treg conversion into inflammatory cytokine producing effector T cells with the same specificity,[35,36] microchimeric fetal cells also have the dangerous potential for sensitizing responses that may trigger autoimmunity.[37-39] This is analogous to pathological responses to microchimeric maternal cells in offspring with various diverse autoimmune disorders including diabetes,[40] biliary atresia,[41] and dermatomyositis.[42] Therefore, establishing the molecular signals that reinforce Treg differentiation stability are of equally high importance and priority.

Nevertheless, applied to the devastating complications in human pregnancy that stem from underlying defects in fetal tolerance (preeclampsia, prematurity, miscarriage), basic investigation on the fundamental biology of $CD4^+$ T cells and memory features for protective regulatory subsets provides renewed hope for new, more efficacious therapeutic approaches. In turn, given the striking parallels between Treg and effector $CD4^+$ T cell memory, unraveling how maternal Treg memory is sustained will likely also provide critical insights for priming more durable effector T cells with pathogen specificity for augmenting host defense against infection.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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