Uncovering the true phenotype of pathogen-reactive innate-like B cells

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Introduction

What are innate-like B cells?

- There are two separate lineages of B cells:
  - Conventional B-2 cells: Part of the adaptive immune response to antigens
  - Innate-like B cells (B-1 cells):
    - Contribute to immune responses in an innate-like fashion by making"natural" antibodies without prior antigen exposure
    - Two subsets differentiated based on CD5 expression: **B-1a (CD5+)** and **B-1b (CD5-)**
- B-1 cells have homeostatic and immune defense functions
  - Natural antibody secretion (mainly IgM)
    - Secreted throughout life
    - Can bind to both self-antigen and to pathogens
  - Response to pathogens—both antibody production and accumulation of B-1 cells at the site of infection
    - Previous studies found IgM is secreted by **B-1b cells** in response to infections, including *Salmonella typhimurium*[1], *Borrelia hermsii*[2], and *Streptococcus pneumoniae*[3].

B-1 cells during influenza infection

- We previously reported that **B-1a** cells, but not **B-1b** cells, migrate from the pleural cavity and accumulate in the draining (mediastinal) lymph node (MedLN) in response to type 1 interferons, secreted after influenza infection[4,5].
- Despite this, our preliminary data indicate that the main IgM-secreting B-1 cells in the MedLN after infection are **CD5-**.

B-1 cells are protective during many infections. Identifying which B-1 cells respond after activation will contribute to the development of better prevention and treatment options for influenza and other infections.

Study Goals

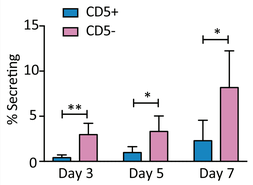
Hypothesis:

B-1a cells activated by influenza infection lose CD5 expression and become "B-1b-like" IgM-secreting cells.

Aims:

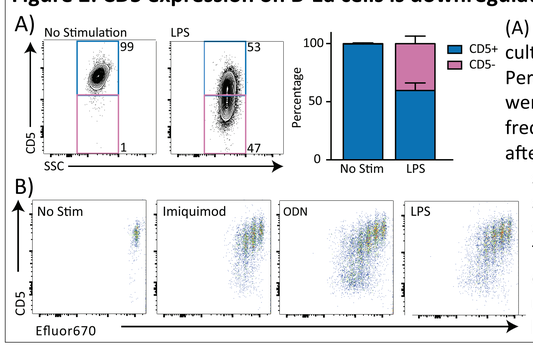
1. Examine whether B-1a cells in the regional lymph nodes (MedLN) downregulate CD5 expression after influenza infection.
2. Examine IgM secretion by B-1a cells in the MedLN after influenza infection.
3. Determine B-1a cells responses to Salmonella infection.

Figure 1: CD5- B-1 cells form more IgM-secreting cells than CD5+ B-1 cells in the Mediastinal LN after infection.



CD5+ and CD5- B-1 cells were sorted from the mediastinal lymph node on days 3, 5, and 7 after influenza infection. Cells were plated on ELISPOT plates to determine the frequency of B-1-derived IgM secreting cell formation. CD5- B-1 cells formed IgM secreting cells at a higher frequency than CD5+ B-1 cells.

Figure 2: CD5 expression on B-1a cells is downregulated *in vitro* after stimulation.



(A) B-1a cells were FACS sorted and cultured with LPS stimulation. Percentages of CD5+ and CD5- cells were determined on day 3. The frequency of CD5- cells was increased after LPS. (B) B-1a cells were FACS sorted and either un-stimulated or stimulated with a TLR7 (Imiquimod) agonist, a TLR9 agonist (ODNs), or a TLR4 agonist (LPS). The stimulated cells down-regulate CD5 as they undergo cell division, as indicated by loss of Efluor670.

Figure 4: Mice receiving only B-1a cells formed B-1-derived IgM secreting cells after infection

Neonatal chimeric mice were infected intranasally with influenza virus. Seven days after infection, mediastinal lymph nodes (MedLN) were collected. B-1-derived IgM antibody secreting cell (ASC) formation by measured by IgM^b-specific ELISPOT. Mice given >50% B-1a cells formed more B-1-derived IgM secreting cells than mice given mainly B-1b cells.

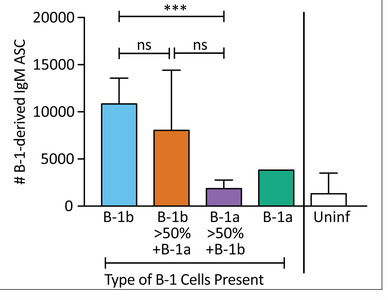
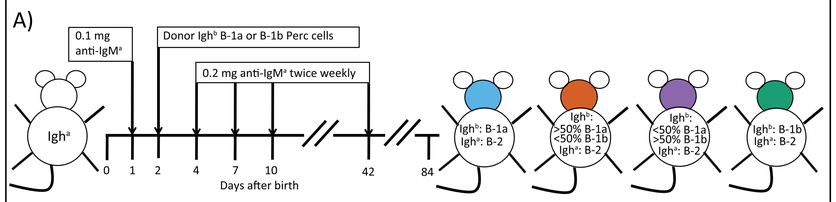
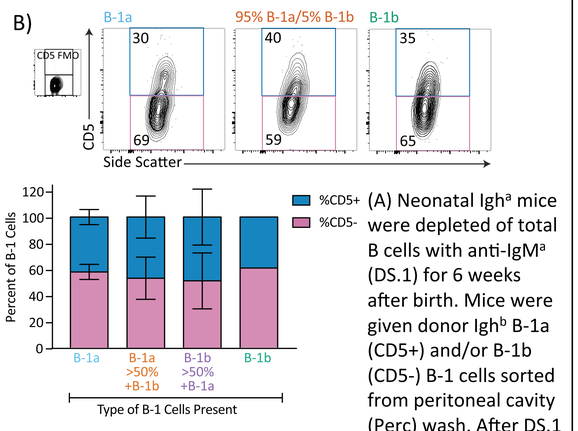


Figure 3: B-1a cells downregulate CD5 expression after influenza infection.

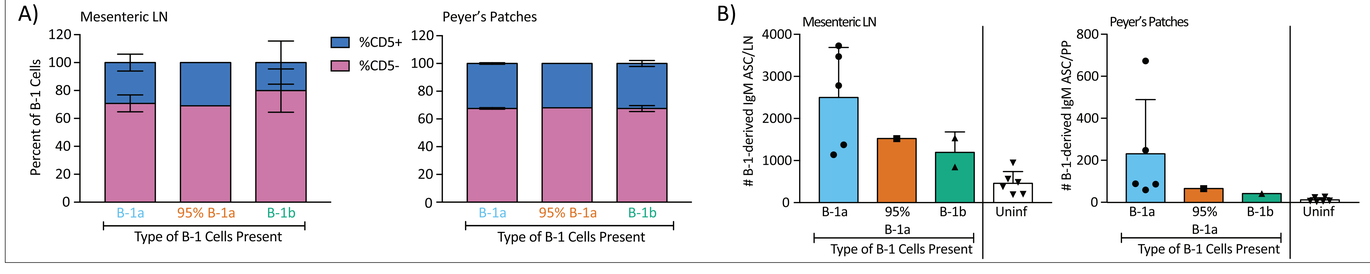




(A) Neonatal Igh^a mice were depleted of total B cells with anti-IgM^a (DS.1) for 6 weeks after birth. Mice were given donor Igh^b B-1a (CD5+) and/or B-1b (CD5-) B-1 cells sorted from peritoneal cavity (Perc) wash. After DS.1 treatment, mice were allowed to rest for 6 weeks to regenerate conventional B (B-2) cells. B-1 cells and B-1-derived IgM are IgM^b+, while B-2 cells and B-2-derived IgM are IgM^a+.
(B) Neonatal chimeric mice were infected intranasally with influenza virus. Seven days after infection, mediastinal lymph nodes (MedLN) were collected and examined by flow cytometry. B-1 cells ("Dump" negative IgM^b+) were examined for CD5 expression. Mice were grouped based on whether they received only B-1a, only B-1b, >50% B-1a, or >50% B-1b cells. Note that all groups had similar % of B-1a and B-1b cells in the MedLN after influenza infection.

Figure 5: B-1a cells down-regulate CD5 and start secreting IgM after *Salmonella typhimurium* infection.

(A) Neonatal chimeras with either only B-1a cells, only B-1b cells, or 95% B-1a + 5% B-1b cells were infected orally with *Salmonella typhimurium*. Five days after initial exposure, mesenteric lymph nodes and Peyer's Patches were collected and examined by Flow Cytometry and ELISPOT. B-1 cells ("Dump" negative IgM^b+) were examined for CD5 expression. Chimeric mice given only B-1a cells had significant populations of CD5- B-1 cells in their MesLN and Peyer's Patches after infection, similar to the populations seen in the other two groups. (B) IgM secretion by B-1 cells in the MesLN and Peyer's Patches of each group was also examined. Mice given only B-1a cells formed more B-1-derived IgM antibody secreting cells (ASC) than mice given B-1b cells.



Summary and Discussion

The results support our hypothesis that activated B-1a cells downregulate CD5 to become differentiated CD5 negative"B-1b"-like IgM-secreting cells after infection.

1) We found that mice receiving only B-1a cells have both CD5+ and CD5- B-1 cells in their lymph node after infection, suggesting a loss of CD5 on B-1 cells.

2) B-1a cells were able to form B-1-derived IgM secreting cells more effectively than B-1b cells in MedLN after influenza infection. Since we showed previously that CD5- B-1 cells are the main source of IgM after infection, this demonstrates that these CD5- B-1 secreting cells are likely B-1a-derived.

3) CD5-downregulation and IgM secretion by CD5 negative B-1 cells occurs also after Salmonella infection.

Several other infectious disease models have found CD5- B-1b cells to be the major source of infection-induced IgM. We re-examined one (Salmonella) and determined that the responding cells were initially B-1a cells that lost CD5.

Our studies increase understanding on the B-1 subsets that contribute to infection-induced immunity and thereby provide basic information important for the development of future vaccines or prophylaxes aimed at boosting immunity.

Finally, our findings call into question the dogma that B-1a and B-1b cells are two distinct B cell subpopulations. Instead, we propose that at least some CD5- B-1b cells are former CD5+ B-1a cells that have undergone activation.

Future Directions

We are repeating the Salmonella infections in chimeric mice to confirm our data.
We plan to examine the response of B-1 cells to OmpD, a Salmonella protein against which CD5- B-1 cells have been reported to generate protective antibodies.

References & Acknowledgements

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Funding: NIH grants NIH/NIAID R01AI051354, R01AI085568 and U19AI109962 and T-32 AI060555