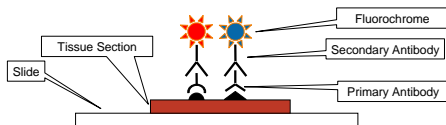


Introduction

- T lymphocytes recognize foreign antigens presented by professional antigen presenting cells such as dendritic cells (DCs). T cells are constantly interacting with DCs in search for the presence of the “foreign” antigens within the secondary lymphoid tissues such as lymph nodes and spleen. The recognition process is critical for mounting protective immunity against pathogens and for establishing memory T cell responses.
- DCs are the major cell types capable of activating naive T cells by presenting peptide antigens as well as by providing additional signals (co-stimulations) necessary for an optimal T cell activation. DCs are also highly efficient in uptaking antigens and presenting them to the interacting T cells. Most DCs express high level of CD11c on the surface.
- In this research we attempt to visualize interactions between T lymphocytes and DCs in situ using a laser scanning confocal microscope.

Methods

- An immunofluorescent technique was used to visualize interactions between T lymphocytes and DCs within the secondary lymphoid tissues.
- Purified antibodies specific for B cells (rat anti-mouse B220) and DCs (hamster anti-mouse CD11c) were used. In order to visualize and amplify the signals, fluorescently conjugated secondary antibodies (Alexa 568 conjugated goat anti-rat Igs and Cy5 conjugated goat anti-hamster Igs) were used. In some experiments, anti-BrdU antibody that stain proliferating cells was used.
- Each fluorochrome, when exposed to a laser, is excited at a certain wavelength. Leica TCS-SP spectral laser scanning confocal microscope was used to acquire images.



Experimental Procedures

- Preparations:**
5µm sections of spleen and lymph nodes are frozen in -80°C until needed.
- Fixation**
4% Paraformaldehyde was used to fix tissue sections (10 minutes)
- Wash (10 minutes)**
The slides are extensively washed in PBS between every step.
- Denaturing (for BrdU staining)**
 - Incubate in 1M HCl on ice (10 min)
 - Incubate in 2M HCl at room temp (10 min)
 - Incubate in 2M HCl at 37°C (20 min)
 - Incubate in 0.1M Borate Buffer (10 min)
 - Wash (10 min)
- Blocking**
Blocking is a step used to prevent nonspecific binding of antibodies on the tissue.
- Wash (10 minutes)**
- Primary Antibody Incubation**
Primary antibodies are added. The primary antibodies bind directly to surface proteins located on the cells. (2 Hours or Overnight)
- Wash (10 minutes)**
- Secondary Antibody Incubation**
Secondary antibodies specific for the primary antibody (as described in the Method section) are added. (1 hour)
- Wash (10 minutes)**
- Mounting**
Vectashield with DAPI is used when mounting cells. DAPI stains nucleus of cells. This is important to locate tissues on slides.

Results

Lymph Node (40x)
Red= B220
Blue= CD11c

This image shows localization of DCs and B cells. DCs are primarily located in T cell areas. (outside B220+ B cell follicles) (Fig 1)

Lymph Node (40x)
Blue= CD11c
Not DENATURED

Lymph Node (40x)
Blue= CD11c
DENATURED

Lymph Node (40x)
Blue= CD11c-Bio
DENATURED

When the tissues were stained for BrdU and CD11c, we were not able to obtain CD11c signals. Further experiments revealed that the denaturing process interferes with CD11c goat anti-hamster binding. This problem was resolved by using a biotinylated anti-CD11c. (Fig 2)

Lymph Node (40x)
Blue = CD11c-Bio
Red = BrdU
Green = B220

•BrdU+ proliferating cells exist through out the lymph node. (Fig 3)

Spleen (40x)
Green = B220
Blue= CD11c

•Localization of B220+ B cells and CD11c+ DCs in the spleen. (Fig 4)

Conclusions

- Procedural Conclusions**
 - The binding of hamster anti CD11c with CY5 anti hamster antibodies were impaired by the antigen retrieval process used to reveal proliferating cells. The use of CD11c-biotinayed followed by streptavidin-CY5 improved the signal. (Fig 2)
- Scientific Conclusions**
 - B cells exist in well defined clusters called B cell follicles in the lymph nodes. (Fig 1)
 - T cells are located in the periarteriolar lymphoid sheath. (Fig 1)
 - Throughout the lymph nodes proliferating cells were found (BrdU+). (Fig 3)

Future Approaches

- Interaction between DCs and adoptively transferred T cells will be examined.
- Different strains of recipient mice (wild type versus lymphopenic TCR B -/-) will be used to examine the roles of peripheral T cells in affecting T-DC interaction.