TH17 cells contribute to uveitis and scleritis and are inhibited by IL-27/STAT1 in the retina. (4) Confocal microscopy

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Abstract

Introduction

*Cheng-Rong Yu prepared this manuscript and should have been the first author. This protocol is for histology and immunohistochemistry. Other protocols related to our Nature Medicine paper can be found here: "Detection of TH17 cells in human blood":http://www.natureprotocols.com/2007/08/30/th17_cells_contribute_to_uveit.php "Western Blot Analysis":http://www.natureprotocols.com/2007/08/30/th17_cells_contribute_to_uveit_2.php "Analysis by confocal microscopy":http://www.natureprotocols.com/2007/08/31/th17_cells_contribute_to_uveit_3.php "Chromatin immunoprecipitation":http://www.natureprotocols.com/2007/08/31/th17_cells_contribute_to_uveit_4.php "Retinal cell isolation":http://www.natureprotocols.com/2007/08/31/th17_cells_contribute_to_uveit_5.php

Reagents

- Immunolabeling buffer \ (1X PBS, 0.5% BSA, 0.2% Tween-20, 0.05% NaN₃, pH 7.3).
- Alexa 488 conjugated goat anti-rabbit secondary antibody \ (Invitrogen, Carlsbad, CA)
- DAPI \ (1ug/ml) \ (Invitrogen).
- Rabbit polyclonal IL-27p28 antibody \ (IMG-5096A-Imgenex, San Diego, CA)
- Normal goat serum • Vectashield mounting medium • Tissue-Tek OCT compound \ (Sakura Finetek, Torrance, CA) • on SuperFrost/ Plus slides \ (Fisher Scientific, Pittsburgh, PA) • Anti-fading agent \ (Gel/Mount, BioMeda, Foster City, CA)

Equipment

- Leica SP2 laser scanning confocal microscope \ (Leica Microsystems, Exton, PA)
- Leica 3060S cryostat

Procedure

****Histology and immunohistochemical analysis****

- 1| Euthanize adult mice \ (C57bl/6 strain) by CO₂ and embed globes in cryomolds containing Tissue-Tek OCT compound and frozen on dry ice.
- 2| Cut frozen sections \ (10 µm thickness) with a Leica 3060S cryostat and collect on SuperFrost/ Plus slides and store at -70°C.
- 3| Prior to immunolabeling, allow slides to air dry under vacuum for 20 minutes.
- 4| Fix sections for 10 minutes in 4% formaldehyde in 1X PBS, wash repeatedly in 1X PBS, prior to blocking in 5% normal goat serum in immunolabeling buffer
- 5| For paraffin embedded sections fix eyes in 10% Formalin \ (overnight)
- 6| Embed globe in paraffin and cut 5µm sections with a microtome
- 7| Paraffinize sections used for antibody staining as follows:
 - Incubate in xylene for 3 minutes each \ (3X).
 - Incubate in 100% ethanol for 2 minutes \ (2X).
 - Hydrate by placing slide in 95% ethanol for 2 minutes \ (2X)
 - Then 70% ethanol for 2 minutes \ (2X)
 - Then 50% ethanol for 2 minutes \ (2X)
 - Then 30% ethanol for 2 minutes \ (2X)
- 8| Transfer sections to distilled water.
- 9| Block with normal rabbit serum for 1 hr.
- 9| Dilute rabbit

polyclonal antibody to IL-27 to 5 and 10µg/ml in immunolabeling buffer (stock antibody =500ug/ml/ working dilutions of 1:50 and 1:100). 10| Incubate sections in diluted primary antibody overnight at 4°C in a humidified chamber. 11| Rinse sections in immunolabeling buffer (3X) 12| Then incubate in Alexa 568 conjugated goat anti-rabbit secondary antibody containing DAPI (1µg/ml) for 1hr at room temperature in an opaque chamber. 13| Wash sections in immunolabeling buffer (3X) 14| Then mount in anti-fading agent and cover with cover-slip. Primary antibody was omitted from sections used as negative controls. 15| Examine sections on a Leica SP2 laser scanning confocal microscope 16| All images were collected under identical PMT detector settings.

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