

Quantification of tissue eosinophils.

Tissue eosinophils are detected using an immunohistochemical stain against murine eosinophilic major basic protein (MBP). Endogenous peroxidase activity is quenched using a 0.3% hydrogen peroxide in methanol solution. Tissue is subjected to pepsin for 10 minutes at 37°C (DIGEST-ALL™ 3, Zymed Laboratories Inc.). Non-specific binding is blocked using 3% goat serum in PBS for 2 hours at room temperature followed by the addition of rabbit anti-murine MBP (~1:8000) primary antibody (kindly provided by Dr. Jamie Lee, Mayo Clinic Scottsdale, AZ), which is allowed to incubate overnight at 4°C. Slides are incubated with biotinylated goat anti-rabbit (1:250) secondary antibody for 40 minutes at room temperature and then incubated with an avidin-peroxidase complex for 30 minutes (Vector Laboratories). Development of peroxidase reaction is achieved by incubating slides with nickel diaminobenzidine-cobalt chloride solution (Vector Laboratories) for 4 minutes at room temperature and then counter-stained with nuclear fast red. Quantification of positive cells is performed using ImagePro Plus imaging software and results are reported as immunoreactive cells per square millimeter.