Mast cell immunohistochemistry: non-immunological immunostaining mediated by non-specific F(ab′)2-mast cell secretory granule interaction

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Summary

During investigations of murine and human mast cell immunoreactivity with potential anti-interleukin-4 antibodies, non-specific, non-immunological labelling of mouse and human mast cells became apparent. Non-specific, non-immunological labelling was identified by (i) immunolabelling of mast cells when using control isotype primary antibodies, (ii) ability of conjugated secondary antibodies to label mast cells without prior mast cell exposure to a primary antibody, (iii) extinction of the non-specific labelling and retention of specific labelling when the pH of the diluting and washing buffers is shifted from pH 7.2 to pH 6.0, and (iv) reduction/extinction of the labelling when the antibodies are pre-incubated with soluble heparin prior to immunostaining. The site of the reactivity on the electron microscope level was shown to be confined to the mast cell secretory granules. The results of this study support the hypothesis that non-specific labelling of mast cells results from an ionic interaction between the F(ab′)2 segments of antibodies and the heparin constituent of the mast cell secretory granules. This study points out the necessity of stringent controls when using immunohistochemistry to determine mast cell reactivity to various antibodies.

Introduction

The secretory granules of cutaneous mast cells contain heparin, a glycosaminoglycan containing numerous sulphate and carboxyl groups which impart a highly negative charge to the molecule. This highly negative charge would favour ionic binding of heparin to molecules having a high isoelectric point (pI > 10) such as avidin, immunoglobulins and certain enzymes (Bussolati & Gugliotta, 1983; Duhamel & Whitehead, 1990).

Non-specific binding of primary antibodies against regulatory peptides in human tissue mast cells has been reported (Scheck et al., 1987; Horny et al., 1988). More recently, it has been postulated that the secretory granules of human tissue mast cells bind certain primary antibodies against regulatory peptides by a cation-exchange mechanism involving ionic interactions between the mast cell granules and the positively charged F(ab′)2, and/or the Fc segments of the immunoglobulins (Ruck et al., 1990). The ability of mast cell secretory granules and the secretory granules of various endocrine cells and nerves to act as cation exchangers with inorganic ions and biogenic amines had been documented (Uvnas et al., 1970, 1985, 1986).

A number of possible explanations have been advanced to account for non-specific binding of immunoglobulins to endocrine cells. These include: complement mediated binding (Buffle et al., 1979a,b), Fc-receptor-mediated binding (Pouplard et al., 1976) and ionic interactions between constituents of the endocrine cell secretory granules and immunoglobulins (Grube, 1980). Similar mechanisms may be proposed to account for non-specific immunoglobulin–mast cell interactions.

Non-specific, non-immunological labelling of mast cells became apparent during our studies to characterize mast cell reactivity with various antibodies. In this study, we investigated whether non-specific, non-immunological labelling of mast cells by immunoglobulins is a general phenomenon, and whether the non-specificity is Fc- or F(ab′)2-mediated; we also investigated the localization of the interaction in normal human skin mast cells at the electron-microscopy level.
Materials and methods

OCT compound was from Miles Inc. (Elkhart, IN). mAb, rat-anti-murine-IL-4 ascites (11B11) and anti-murine-IL-4 (91-1) rabbit serum were a generous gift from Dr Junichi Ohara. mAb, rat-anti-mouse-T cell (Thy 1.2) was from Becton-Dickinson (San Jose, CA). mAb, rat-anti-mouse mast cell and macrophage (B5A.2) was a generous gift from Dr Howard Katz. Avidin–Texas Red (a secondary non-immunoglobulin conjugate), and alkaline phosphatase-conjugated secondary antibody (goat-anti-rabbit-lgs (GARIG), goat-anti-rat-lgs, and F(ab')2 segments) were from Tago Immunologicals (Burlingame, CA). Goat-anti-rabbit-lgs conjugated to 10 nm gold particles was from ICN Biomedicals Inc. (Costa Mesa, CA). IgG1, isotype control antibody (MOPC-21), bovine serum albumin (BSA), reagent-grade acetone, collagenase, trypsin, Trizma base, Tris–HCl and alkaline phosphatase substrate were from Sigma (St Louis, MO). Heparin derived from porcine intestinal mucosa (1000 USP units ml⁻¹) was from SoloPak Labs (Franklin Park, IL). Epon was from Ted Pella Inc. (Redding, CA). All other chemicals used were of at least reagent-grade specification.

Murine dermal cell cytocentrifugation preparations (cytopins)

Murine ears are normally a rich source of connective tissue mast cells and were used, as follows, to provide cell suspensions that were enriched in mast cells. Excised mouse ears were incubated in 0.25% trypsin for 2 h at 37°C. The epidermis was then teased apart from the dermis and the dermis was incubated in 2.5 mg ml⁻¹ collagenase for 3 h at 37°C. The mixture was shaken and allowed to stand at ambient temperature for 2–3 min to allow large clumps to settle. The supernatant was then aspirated and centrifuged for 5 min at 350 g. The pellet was resuspended, and mast cells were counted using a haemacytometer and Toluidine Blue (1%, pH 3.2) staining. Allquots of the suspension, which contained all types of connective tissue cells, were then cytocentrifuged onto slides (at a concentration that provided approximately 50 mast cells/slide), air dried, treated with reagent-grade acetone for 10 min, and stored at −70°C until immunohistochemistry was to be performed.

Murine ears

Excised murine ears were embedded in OCT compound, quick-frozen in liquid nitrogen, and stored at −70°C prior to sectioning. Thick sections (6–8 μm) were cut with a cryostat, flash-dried in a 60°C oven, and immunostained as described.

Immunohistochemistry

Immunohistochemical staining was performed using a two-step alkaline phosphatase procedure as previously described (Seibold et al., 1991; Claman et al., 1991). We used a Shandon Sequenza immunostainer (Shandon, PA) at ambient temperature for staining with primary antibody (60 min) and secondary antibody (30 min) with appropriate washes and alkaline phosphatase and Haematoxylin stains.

Avidin–Texas Red staining was performed as previously described (Giorno et al., 1987). The method is similar to that described above except that it contains avidin–Texas Red but no alkaline phosphatase or Haematoxylin. Avidin–Texas Red staining was visualized with a fluorescence microscope.

Blocking

Cytopsin samples of the murine ear dermal digests and thick sections of murine ear were incubated with BSA (0.2–2%) for 30 min prior to immunostaining procedures. Additionally, antibodies were incubated for 30 min with heparin (1000 U ml⁻¹ in TBS prior to immunostaining). BSA was chosen to determine whether the non-specificity was a result of general non-specific-type protein–protein interactions. Since we felt that it was more likely that the non-specific labelling was a result of an ionic interaction between heparin and the antibodies, we used pretreatment of the antibodies with heparin to determine whether subsequent antibody labelling could be reduced.

pH acidification

Cytopsin preparations and thick sections of ears were immunostained as described, with the exception that the pH of all diluting and washing buffers was adjusted to 6.0.

Electron microscopy

Human skin was obtained via a 5 mm punch biopsy of forearm skin and fixed for 2–4 h in 3% paraformaldehyde and 0.025% glutaraldehyde in 0.05 M PBS (pH 7.4) at 4°C. Tissue was then dehydrated and processed using standard electron-microscopy techniques and embedded in Epon. Ultrathin sections (silver/grey) were deplasticized with saturated sodium ethoxide for 4 min prior to tissue exposure with antibodies (Mar & Wight, 1988).

Deplasticized grids were first hydrated and then exposed to goat-anti-rabbit-IgG conjugated to 10 nm colloidal gold particles in 0.05 M Tris-buffered PBS at 4°C overnight. Grids were then treated, after washing, with 2% glutaraldehyde for 10 min, stained with 1% osmium tetroxide, dehydrated through ascending grades of ethanol, and re-emerged in 2% Epon. Grids were counterstained with 4% aqueous uranyl acetate and lead citrate and examined in an electron microscope (Philips CM-10, 80 kV).

Fig. 1. Immunoreactivity of mast cells. Toluidine Blue staining of murine cytopins demonstrating a typical mast cell (arrowheads, A). Normal rabbit serum as control serum exhibiting non-specific immunobelling of mast cell with alkaline phosphatase–GARIG secondary in cytopins (arrowheads, B). Labelling of mast cells by secondary antibody conjugate (alkaline phosphatase–GARIG) without prior mast cell exposure to a primary antibody in cytopins (C) and in whole murine ear (D). Thy 1.2 labelling of T-cells at pH 7.4 (E) and retention of specific staining of T-cells (Thy 1.2) when the pH of the diluting and washing buffers is adjusted to 6.0 (F). Electron micrograph of human dermal mast cell displaying labelling by a secondary antibody conjugate (GARIG conjugated to 10 nm gold particles) without prior tissue exposure to a primary antibody (G). Note the localization of the gold particles over the secretory granules of the mast cells and the relative lack of gold particles in other areas. A–F, original magnification ×400; G, original magnification ×8900. GARIG = goat-anti-rabbit-IgG.