Histamine Release from Human Leukocytes When Stimulated by *Mycoplasma salivarium*

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Incubated mixtures of *Mycoplasma salivarium* and nonsensitized mixed leukocytes released histamine. Spectrophotofluorometric detection of histamine occurred at physiological temperature and pH independent of complement and specific antiserum.

As early as the 1950s, it was postulated that the inflammatory response of tissue is mediated by histamine (5). Numerous antigenic substances have been shown to stimulate the release of histamine from leukocytes (7, 11-13, 15, 21). Each of these investigations found natural sensitization or preincubation of the leukocytes with sera from sensitive donors or anti-immunoglobulin E essential to cause leukocytic histamine release. Recently, lysates of mixed human leukocytic suspensions were found to release histamine from intact human leukocytes (8). Basophilic granulocytes are the major source of histamine in humans (6, 7, 17). Inflammatory reactions (9) and traumatic responses (19) cause extravascular migrations of basophils to sites of injury. These phenomena invite speculation that *Mycoplasma salivarium*, as it is phagocytized by human leukocytes (16), stimulates histamine release from leukocytes that are known to migrate to inflamed periodontal tissues (22). This is important since observations indicate *M. salivarium* to be the predominant mycoplasmal sulcular inhabitant in inflammatory gingivitis (4, 18). The present report indicates that prior leukocytic sensitization is not required for histamine release. In addition, explanations for this observation are given.

All experiments were performed with *M. salivarium* strain PG 20, obtained from the National Institutes of Health, Bethesda, Md. Confirmation of species was accomplished by the growth inhibition method described by Clyde (2), using paper disks saturated with homologous and heterologous antimycoplasmal sera (BBL).

The medium used in culturing the mycoplasmas contained 8 volumes of PPLO broth or agar (BBL), 1 volume of agamma horse serum (BBL), and 1 volume of a 1% Albimini Laboratories yeast autolysate solution with 500 U of penicillin per ml and 250 mg of thallium acetate per liter. The microorganisms were harvested during logarithmic growth phase by centrifugation at 27,000 × g for 20 min at 4 C. The mycoplasmas were washed twice and resuspended in phosphate-buffered saline (pH 7.2).

Enumeration and viability of mycoplasmas were determined from broth, phosphate-buffered saline suspensions, or incubation mixtures taken at various times, serially diluted in PPLO broth free of additives and supplements, and then plated on agar. The inoculated agar was incubated under CO₂ at 37 C for 48 h and was then inspected for colony-forming units (CFU) of *M. salivarium* per milliliter by using a Unitrone inverted microscope at ×75 magnification.

Human blood without serum growth-inhibiting antibody to *M. salivarium* was collected in plastic test tubes containing 10 U of heparin per ml. Three normal males having an average age of 23.7 years and without clinical evidence of periodontal disease were the blood donors. Tests of growth inhibition were performed on these sera by a modified method of Bailey et al. (1) as reported by Kumagai et al. (10). The tested sera were considered without antibody when CFU of *M. salivarium* per milliliter were greater than nine-tenths of the control. Theuffy coat and plasma were aspirated after sedimentation of erythrocytes with a plastic pipette and centrifuged at 180 × g at 4 C for 8 min. The button of cells containing the leukocytes was gently washed twice and resuspended in tris(hydroxymethyl)aminomethane buffer at pH 7.3 at 37 C to 1.5 ± 0.3 × 10⁷ leukocytes per ml. Washed leukocytes were considered nonsensitized if they were derived from human blood in
which the serum was unable to prevent colony formation of \textit{M. salivarium}. The characteristics of cells were checked by microscopy.

The preparation of incubation mixtures in polycarbonate tubes consisted of 1 ml of leukocytes, 1 ml of mycoplasmas of various concentrations, and 2 ml of buffer and essentially followed the protocol of Lichtenstein and Osler (13). The preparations and controls were incubated for 2 h at 37°C in an environmental shaker rotating at 150 rpm. After incubation, the reactive mixtures were centrifuged to sediment both mycoplasmas and leukocytes and each tube was deproteinized except a tube containing only leukocytes and buffer, which supplied the value for the total (complete) extractable histamine.

Due to variations in the histamine content of cells derived from different donors, the results were plotted in terms of percentage of histamine released. The percentage of histamine released is equal to the specific relative fluorescent intensity less the spontaneous histamine release and reagent fluorescence from a control tube (blank) of leukocytes without mycoplasmas divided by the fluorescence from the "complete" less the "blank" times 100.

Leukocytes remained viable for the duration of the experiments, but the CFU of \textit{M. salivarium} per milliliter were fewer at the conclusion than at the beginning of experimentation. The leukocytes released part of their histamine content in the presence of 5, 50, or 1000 mycoplasmas per leukocyte. Increased numbers of \textit{M. salivarium} per leukocyte resulted in greater histamine release, and the dose response curve is presented in Fig. 1. The detected histamine release with 5 mycoplasmas per leukocyte was approximately one-half that detected when the ratio was increased to 100 mycoplasmas per leukocyte.

This preliminary study shows that \textit{M. salivarium} stimulated histamine release from mixed human nonsensitized leukocytes. Further investigation is needed and will be initiated to elucidate this phenomenon, but preliminary explanations are required at this time.

This effect may be explained by nonspecific sensitivity and finds supportive evidence in the studies of May and Williams (14). Their investigations of unrelated allergens indicated nonspecific sensitivity by degranulating basophils in releasing histamine. Another explanation for the release of histamine may be caused by residual or undetected immunological reactions. The leukocytes used in these experiments were derived from donors whose sera did not inhibit colony formation by \textit{M. salivarium} on agar. This method, although used for confirmation of presence of mycoplasmal species (1, 2), may be insensitive to small amounts of antigen serum. Reasonable explication for the detected histamine in this system may reside in factors associated with phagocytosis. The basis for this postulate is derived from ultrastructure studies by Parkinson and Carter (16), which show phagocytosis of \textit{M. salivarium} with accompanying degranulation of leukocytes. During phagocytosis, Cohn and Hirsch (3) observed the release of lysosomal enzymes from cytoplasmic granules. Kelly et al. (8) demonstrated that lysosomal lysates from leukocytes contained significant histamine-releasing activity for leukocytes. The 2-h incubation of the reaction mixture allowed in this study provides adequate time for the release of lysosomal content (16). Therefore, it may be that the detected histamine occurred secondarily to factors associated with in vitro phagocytosis of \textit{M. salivarium}.

Since phagocytosis of \textit{M. salivarium} decreases the number of mycoplasmas, all detectable levels of histamine would have to be considered with respect to the phagocytic process. The lower percentage of histamine released with fewer numbers of mycoplasmal organisms is presumably a dilution factor. At any concentration of mycoplasmas to leukocyte, histamine might be released but remain unavailable for
assay because of instability or destruction.

This study is valuable because the in vitro system used to detect histamine release involved human leukocytes. This eliminated difficulties in crossing species and examined the effects of a known human oral microbial inhabitant on human cells. It is not untenable that released histamine from a few leukocytes in vivo might increase concentrations sufficient to mobilize additional histamine from neighboring blood leukocytes and potentiate the inflammatory process.

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LITERATURE CITED


