Journal of Clinical Microbiology and Antimicrobials

OPEN ACCESS Freely available online

Commentary

Purification of Human Eosinophil Contain A Powerful Neurotoxin that Causes Selective Neuronal

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INTRODUCTION

Eosinophils contain a powerful neurotoxin that can severely damage myelinated neurons in experimental animals. M. H. Gordon described this neurotoxic reaction in 1932; it is now known as the "Gordon phenomenon" in his honor. At present, the characteristic and easily recognizable neurologic and histopathologic changes that occur after injection of eosinophil extracts into laboratory animals provide the only available assay for the eosinophil-derived neurotoxin (EDN). The neurologic abnormalities include stiffness and ataxia, progressing to severe paralysis. Histopathologically, there is selective and widespread loss of Purkinje cells and severe spongiform degeneration in the white matter of the cerebellum, brain stem, and spinal cord. Purification of Eosinophils. Eosinophils from patient 1 were purified from ascitic fluid that contained a high proportion of eosinophils. Leukocytes from this ascitic fluid were partially purified by centrifugation after layering on Ficoll/Hypaque as described. After hypotonic lysis of erythrocytes, followed by three washes in normal saline, the leukocytes were resuspended in isotonic saline at a concentration of 3.5 × 107 cells per ml and stored at -75°C. This preparation contained 98% eosinophils, 0% neutrophils, and 2% mononuclear cells [1].

Purification of Eosinophil Granules

Eosinophil granules were prepared from the eosinophil-rich leukocyte suspensions obtained from patients 2 and 4 as described in detail elsewhere (13-15). Briefly, cells were gently washed once with ice-cold 0.25 M sucrose, resuspended in cold 0.25 M sucrose, disrupted by vigorous and repeated pipetting through a narrow-bore 10 ml calibrated pipette, and centrifuged to remove unbroken cells and large cell debris. The pellet was resuspended in 0.25 M sucrose and the extraction procedure was repeated four times. The supernatants from these extractions were centrifuged at 13,000 g for 20 min to sediment the granules [2].

Purification of Eosinophil MBP and CLC Proteins

The methods for purification of human MBP are described in detail elsewhere. Briefly, purified eosinophil granules were dissolved in 0.01 M HCl and centrifuged at 40,000 g for 5 min, and the supernatant was fractionated on a 1.2 × 47 cm Sephadex G-50 column equilibrated with 0:025 M sodium acetate buffer, pH 4.2/0.15 M NaCl. The fractions from the third protein peak, which contained MBP, were pooled and MBPP was stabilized by reduction and alkylation of its two sulfhydryl groups Bioassay of Eosinophil Proteins for Neurotoxic Activity [3]. The methods for intrathecal injection of eosinophil extracts and fractions to produce the Gordon phenomenon have been described. Briefly, male New Zealand White rabbits weighing 1.5-3.0 kg were sedated with fentanyl and droperidol (Innovar, McNeil Laboratories, Irvine, CA). A 25-gauge needle was passed into the cisterna magna. The correct position was established by withdrawing a small amount of cerebrospinal fluid, and 0.2 or 0.4 ml of test suspension in PJNaCl was injected. To eliminate possible bias in recording the results of animal experiments, eosinophil fractions were coded so that the observer did not know which fraction each animal had received. After injection, rabbits were examined daily for the typical signs of the neurotoxic reaction. For histologic study of the brain, rabbits were killed by intravenous injection of pentobarbiton [4].

CONCLUSION

The biological and clinical significance of the Gordon phenomenon remains enigmatic. Certainly, eosinophil granules contain a powerful neurotoxin that is capable of damaging and ultimately destroying myelinated axons and neurons. The damage is highly selective in the experimental animal, affecting chiefly white matter of cerebellum and spinal cord. Lesions may occur elsewhere in the brain and peripheral nerves, but these are minor compared with the destruction of white matter in cerebellum and spinal cord. The neurotoxic effect is dose dependent.

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Received: 05-Dec-2022, Manuscript No. JCMA-22-17955;**Editor assigned:** 07-Dec-2022, PreQC No: JCMA-22-17955(PQ); **Reviewed:** 21-Dec-2022, QC No: JCMA-22-17955;**Revised:** 28-Dec-2022, Manuscript No: JCMA-22-17955; **Published:** 04-Jan-2023, DOI: 10.35248/JCMA. 22.6.145.

Citation: Joseph R (2022) Purification of Human Eosinophil Contain A Powerful Neurotoxin that Causes Selective Neuronal. J Clin Microbiol Antimicrob 6:006.