Serum xanthine oxidoreductase: Hydrogen peroxide production rates in mammalian sera

Andrew D. G Van Praagh, *University of Massachusetts Amherst*

Abstract

In the African Cape buffalo, circulating serum xanthine oxidoreductase (XOR) produces trypanotoxic levels of hydrogen peroxide (H_2O_2). Previous, preliminary studies suggest that mammalian serum XOR H_2O_2 production rates ("specific activities") are species- and breed-associated. The research objectives pursued here were: (1) to verify that mammalian serum XOR specific activities were truly species- and breed-associated, and if found to be so (2) to determine if the noted activity differences were a product of translational and/or post-translational mechanism(s). The ultimate intent of this work was to reveal strategies by which trypanosome tolerance might be achieved in susceptible mammals. ^ By a novel kinetic peroxidase assay, developed and used here, it was found that the serum XOR specific activities of Cape buffalo, Holstein and Hereford heifers, Sprague-Dawley rats, and humans were significantly different from one another ($p < 0.025$). A concurrent, comparative analysis of XOR nucleotide/amino acid sequences and tissue-specific transcription rates in Cape buffalo and representative bovine breeds yielded no points of variability that might explain observed activity differences. To determine if such differences were, instead, a product of distinct translational and/or post-translational regulatory mechanism(s), serum XOR was isolated, quantified, and characterized. ^ Two immunoaffinity (IA) chromatography protocols were developed to isolate serum XOR. Mouse monoclonal antibodies directed against cow milk xanthine oxidase (α-CMXO) exhibited a recognition for serum XOR that was limited to bovid species. Polyclonal rabbit α-CMXO antibodies were found to recognize serum XOR from a greater range of mammalian species. However, IA eluent resolution by SDS-PAGE (under reducing conditions) elucidated polypeptide profiles that were species-specific, suggesting the presence of co-eluting contaminants. MALDI-TOF analysis identified the putative contaminants as components of serum IgG and IgM. These immunoglobulins (Igs) were found to exist in complex with the isolated serum XOR or were recognized directly by the polyclonal α-CMXO antibodies of the IA column, in an idiotypic manner. The consequences and implications of such Ig contamination are discussed, specifically with regard to: (1) the future use of α-XOR antibodies in extracting serum XOR, and (2) the possible roles of XOR-Ig complexes in systemic inflammatory pathologies. ^

Subject Area

Pathology|Immunology

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