Measurement of Candidacidal Activity of Specific Leukocyte Types in Mixed Cell Populations

II. Normal and Chronic Granulomatous Disease Eosinophils

ROBERT I. LEHRER
Cancer Research Institute and Department of Medicine, University of California School of Medicine, San Francisco, California 94122

Received for publication 9 February 1971

Normal human eosinophils possess appreciable intrinsic candidacidal activity. The leukocyte microbicidal deficiency of chronic granulomatous disease is manifested by eosinophils as well as by neutrophils and monocytes.

Despite major advances in our understanding of the composition, metabolism, and distribution of eosinophils, considerable uncertainty remains as to their specific functions (1, 3, 5, 19). For example, although their phagocytic activity may at times approach that of neutrophils, their ability to function as microbicides is unknown (1, 4, 21). This report describes the use of a newly developed technique to measure the candidacidal activity of human eosinophils in short-term tissue cultures. As the neutrophils and monocytes of patients with chronic granulomatous disease are known to be deficient in their ability to kill many species of bacteria and fungi (8, 11, 13, 16), the eosinophils of two boys with this disorder were also studied.

MATERIALS AND METHODS

Mixtures of peripheral blood leukocytes, normal group AB serum, and Candida albicans were prepared for these studies as previously described (12). The Candida cells were all ingested within minutes after their addition to the mixtures, mainly by neutrophils and monocytes. The portion of added fungi ingested by eosinophils in these studies ranged from 0.1 to 1.2% of the initial inoculum.

The viability of C. albicans within intact leukocytes was assessed by a technique based on alterations in the shape and staining properties of the organisms after defined periods of intracellular residence (12). After incubation at 37 C for 2.5 hr, samples of the mixtures were transferred to glass slides with a Shandon cytocentrifuge, fixed, and stained with Giemsa. Phagocytic eosinophils were identified and examined until 50 to 100 consecutively encountered intracellular organisms were found and classified as unaltered yeasts, germinated yeasts, or “ghosts.” Unaltered yeasts were those that preserved their spherical shape and retained the homogeneous blue cytoplasmic staining pattern that characterized the live organisms at the time of their addition. Germinated yeasts bore characteristic filamentous pseudogerm tubes, prima facie evidence of intracellular survival and subsequent growth. “Ghosts” were organisms that were devoid of cytoplasmic basophilia due to depletion of their cytoplasmic ribonucleic acid content after death within the phagocyte. In earlier studies of the candidacidal activity of human neutrophils, the percentage of intracellular “ghosts” found was found to correspond closely to the percentage of nonviable organisms as determined by an independent method (12).

RESULTS

Examination of Candida ingested by eosinophils from 14 normal subjects, 9 patients with bacterial or fungal infections, and 5 patients with neoplasia gave virtually identical results. Overall, 42.0 ± 1.7% (mean ± standard error) of the ingested organisms were “ghosts,” 28.5 ± 1.8% had germinated, and 29.5 ± 1.7% were unaltered after 2.5 hr. The viability of the unaltered organisms was not determined; they may have represented live organisms that had not yet formed pseudogerm tubes or nonviable organisms with insufficient cytoplasmic degradation to result in the “ghost” appearance. Tested under comparable conditions and analyzed after 2.5 hr, 28.1 ± 1.8% (mean ± standard error) of Candida within normal human neutrophils (12) and 64.1 ± 2.0% of Candida within normal human monocytes (11) were “ghosts.” In contrast to normal leukocytes, eosinophils from two young boys with chronic granulomatous disease did not kill ingested C. albicans. This was shown by an almost complete absence of intracellular “ghosts” and by the presence of pseudogerm tubes on virtually all of the ingested Candida cells (Fig. 1A and B).

DISCUSSION

Previous studies have suggested that the ability of normal human neutrophils and monocytes to
kill \textit{C. albicans} derives from an interaction between the granule enzyme myeloperoxidase and hydrogen peroxide \((11, 14)\). The defective candidacidal activity of neutrophils and monocytes from subjects with chronic granulomatous disease \((11-13)\) has been attributed to their failure to produce sufficient hydrogen peroxide \((9)\) to activate this system normally after ingestion of microorganisms. Although the abundant peroxidase of eosinophil granules \((3)\) differs structurally from myeloperoxidase \((2, 18)\), it has been shown to enter the phagocytic vacuoles of eosinophils that have ingested zymosan particles \((7)\). Moreover, isolated human eosinophil granules exert candidacidal activity when hydrogen peroxide and iodide are added \((10)\). It is possible, therefore, that hydrogen peroxide activates the candidacidal components of the granules after they enter the phagocytic vacuoles of normal eosinophils. Failure to generate hydrogen peroxide \((6, 15)\) could be responsible for the defective function of eosinophils from patients with chronic granulomatous disease.

What, if any, part impaired eosinophil function plays in the greatly increased susceptibility to infection of patients with chronic granulomatous disease cannot be determined at this time. If eosinophils do play a role as phagocytes in resistance to infection, it would most likely be expressed in sites where they are normally abundant or to which they are attracted by disease processes. Rytömaa found significant numbers of extramedullary eosinophils in the skin, gastrointestinal tract, lungs, and uterus of the adult rat \((17)\), all areas of potential contact with the external microbial flora. Reports in the older literature suggest that local eosinophils can protect tissues from microbial invasion until an influx of neutrophils occurs \((20)\).

Human eosinophils may exceed neutrophils in their intrinsic ability to kill \textit{C. albicans}, a common intestinal saprophyte and occasional pathogen of man; this observation suggests that one function of eosinophils may be to augment the antimicrobial defenses of certain tissues.

\textbf{ACKNOWLEDGMENTS}

I thank Gisela L. Schanklies for skillful technical assistance. This work was supported by Public Health Service research grant CA-11067 from the National Cancer Institute and by Cancer Research funds from the University of California.

\textbf{LITERATURE CITED}


\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{candidacidal_activity_of_human_eosinophils.png}
\caption{Interaction between eosinophils and ingested Candida albicans. (A) Percentage of intracellular Candida classified as "ghosts." (B) Percentage of intracellular Candida with pseudogerm tubes. Symbols: \(\bullet\), studies on eosinophils from normal subjects and patients with infections or neoplasms; \(\bigcirc\) and \(\triangle\), studies on two children with chronic granulomatous disease. Most determinations were made after 2.5 hr; a few, as indicated, were made after 2.0 hr.}
\end{figure}