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Effect of Immunosuppression on the Development of Experimental Hematogenous *Candida* Endophthalmitis

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The induction of neutropenia and immunosuppression by the administration of nitrogen mustard (HN_2) decreased the frequency and altered the morphology of clinically detectable hematogenous *Candida* endophthalmitis in the rabbit model of disseminated candidiasis. Whereas 95% of eyes in rabbits infected with *Candida albicans* without pretreatment with HN_2 developed typical lesions of hematogenous *Candida* endophthalmitis, only 6.2% of eyes in rabbits that had been given 3.0 mg of HN_2 per kg developed clinically detectable endophthalmitis. Lesions that developed in the severely immunocompromised and neutropenic rabbits were small and atypical in appearance. From these data, we conclude that ophthalmoscopic examination may not be a sensitive diagnostic modality for disseminated candidiasis in severely immunocompromised, neutropenic patients.

Endophthalmitis is now a well-recognized complication of candidemia (1, 3, 10). Autopsy studies have demonstrated that 78% of all patients who died with *Candida* endophthalmitis had signs of infection elsewhere (1). However, the precise incidence of endophthalmitis in disseminated candidiasis is unknown. Investigators working in centers where immunocompromised, neutropenic patients with disseminated candidiasis are seen have suggested that hematogenous *Candida* endophthalmitis (HCE) may be a relatively uncommon manifestation of disseminated candidiasis in these patients (J. E. Bennett and J. S. Remington, personal communications).

In a pilot study, we evaluated prospectively a group of presumably immunocompetent, medically complex surgical patients who were receiving parenteral hyperalimentation fluids. Patients were evaluated for candidemia, the development of positive *Candida* precipitins, and the development of chorioretinal lesions consistent with HCE (5). This study has now been extended to include an additional 131 patients. Eight of the 131 additional patients had documented candidemia, and, of these 8 patients, 7 (87.5%) developed chorioretinal lesions clinically consistent with HCE.

Since one of the major differences between surgical and immunocompromised patients is the severity of neutropenia and immunosuppression in the latter, we decided to test the hypothesis that neutropenia and broad-spectrum immunosuppression might decrease the incidence of detectable endophthalmitis in the rabbit model of disseminated candidiasis.

(A portion of this work was presented at the 18th Interscience Conference on Antimicrobial Agents and Chemotherapy, Atlanta, Ga., 1978.)

MATERIALS AND METHODS

Introduction of neutropenia. Neutropenia and broad-spectrum immunosuppression were induced in New Zealand white rabbits by the intravenous administration of mechlorethamine hydrochloride (nitrogen mustard; HN_2) (Mustargen; Merck, Sharp and Dohme, West Point, Pa.). Thirty-two rabbits were divided into three groups of 10 rabbits each. The group that received the largest dose of HN_2 contained 12 rabbits because higher mortality was anticipated in this group. Group 1 received 0.6 mg of HN_2 per kg; group 2 received 1.0 mg/kg; group 3 received 3.0 mg/kg; and an additional 10 rabbits (group 4) served as controls. Mean leukocyte counts were determined before HN_2 treatment and 3 days and 5 days after infection. Leukocyte counts were performed on venous blood that had been drawn into a tube containing ethylenediaminetetraacetic acid. The cells were counted in a hemacytometer. Differential leukocyte determinations and calculated absolute polymorphonuclear leukocyte counts were performed by evaluating a Wright-stained smear of this sample.

Experimental disseminated candidiasis and HCE. Experimental disseminated candidiasis and HCE were established as previously reported (2) by the administration of 1 ml of suspension, containing 5.0×10^5 organisms of a clinical isolate of *C. albicans*, Dye strain, per ml, suspended in brain heart infusion broth (Difco Laboratories, Detroit, Mich.), into the marginal ear vein of all 42 New Zealand white rabbits. The yeasts were injected 6 h after HN_2 administration. In previous studies, we have demonstrated minimal mortality in rabbits with normal host defenses with

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this inoculum size, with a 90 to 100% infection rate (2). Lack of detectable endophthalmitis in uninfected rabbits, in rabbits receiving HN_2 alone, and in rabbits receiving only intravenous brain heart infusion broth has been previously established in our laboratories (2).

Clinical indirect ophthalmoscopic examination. On day 5 after infection, both eyes of all 42 rabbits were dilated using 1% cyclopentolate hydrochloride (Cyclogel; Alcon Laboratories, Fort Worth, Tex.) and 10% phenylephrine hydrochloride (Neosynephrine; Winthrop Laboratories, New York, N.Y.). The fundi were examined with an indirect ophthalmoscope (Frigi-Xonix, Shelton, Conn.). Eyes were scored for the presence or absence of chorioretinal lesions, as well as for the extent of disease, utilizing a scale of zero (no involvement) to 4+ (extensive involvement). A single, small lesion was scored as 1+. An eye containing one to five lesions, each less than the diameter of the optic disk, was scored as 2+. Any lesion greater than one disk diameter in size with associated vitreous haziness was scored as 3+. Massive lesions with nearly complete vitreous involvement were scored as 4+.

Postmortem culture technique. Five days after infection, after ophthalmoscopic examination and blood sampling for neutrophil counts, all animals were killed. Quantitative cultures of chorioretina, vitreous, and kidney were taken from each rabbit. The chorioretina was dissected from the vitreous and the remainder of the ocular contents, placed in brain heart infusion broth, and homogenized with a Tri-R grinder (Tri-R Instruments, Rockwell Center, N.Y.). The homogenates were serially diluted in brain heart infusion broth and plated in brain heart infusion agar. Cultures were incubated at 37°C for 48 h, and the number of colony-forming units per gram of tissue was determined. In a similar fashion, the vitreous tissue was suspended in brain heart infusion broth, homogenized, and cultured in brain heart infusion agar. Sagittal sections of each kidney were weighed, homogenized, and cultured in similar fashion.

Data were analyzed by correlating clinical extent of eye infection with mean leukocyte counts, mean polymorphonuclear leukocyte counts, and postmortem culture results from each of the four groups using chi-square analysis with the Yates correction (11).

RESULTS

Leukocyte counts. The results of the absolute polymorphonuclear leukocyte counts are presented in Fig. 1. Each of the groups of rabbits that received HN_2 had a depressed polymorphonuclear leukocyte count 3 days after infection when compared with controls. However, only the rabbits that received the highest dose of HN_2 (3.0 mg/kg) remained neutropenic 5 days after infection. Results of the absolute polymorphonuclear leukocyte counts paralleled the fluctuation seen in the total leukocyte count in all three groups receiving HN_2.

Ophthalmoscopic examination. Typical lesions of HCE developed in 19 of 20 eyes (95%) in non-HN_2-treated control rabbits. In rabbits that

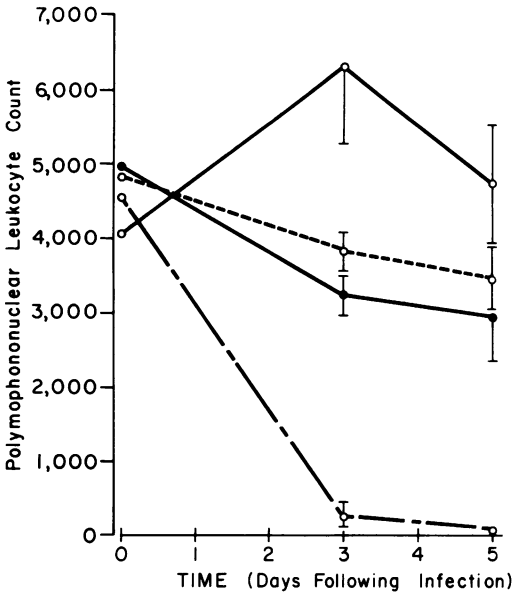

FIG. 1. *Mean absolute polymorphonuclear leukocyte counts in the four groups of rabbits at three periods: before infection, 3 days after infection, and 5 days after infection.* (O——O) *Group 4 (control);* (O·····O) *group 1 (0.6 mg of* HN_2 *per kg);* (●——●) *group 2 (1.0 mg of* HN_2 *per kg);* (O—·—·—O) *group 3 (3.0 mg of* HN_2 *per kg).*

received either 0.6 mg (group 1) or 1.0 mg (group 2) of HN_2 per kg, the incidence of HCE 5 days after infection was 62% (10 of 16 eyes infected in each group). Two rabbits in each of these groups died within 1 day after administration of HN_2. In rabbits that received 3.0 mg of HN_2 per kg (group 3) only one lesion was seen. Sixteen eyes from rabbits in this group could be evaluated at 5 days after infection. (There were four deaths in this group.) The one lesion seen in the group 3 rabbits was much smaller and less well formed when compared to the typical lesion in the infected control rabbits (Fig. 2).

When the groups were compared by chi-square analysis (utilizing the Yates correction), comparing the incidence of HCE in the four groups, groups 1 and 2 were not significantly different (P = not significant) and had significantly more endophthalmitis than the group of rabbits that received the highest dose of HN_2 (group 3) ($P < 0.025$). Both these groups had significantly less eye involvement than controls (group 4) ($P < 0.05$). When the most severely neutropenic, immunocompromised rabbits (group 3) were compared with controls (group 4), the difference in the incidence of HCE was highly significant ($P < 0.0005$).

Postmortem culture results. Results of the

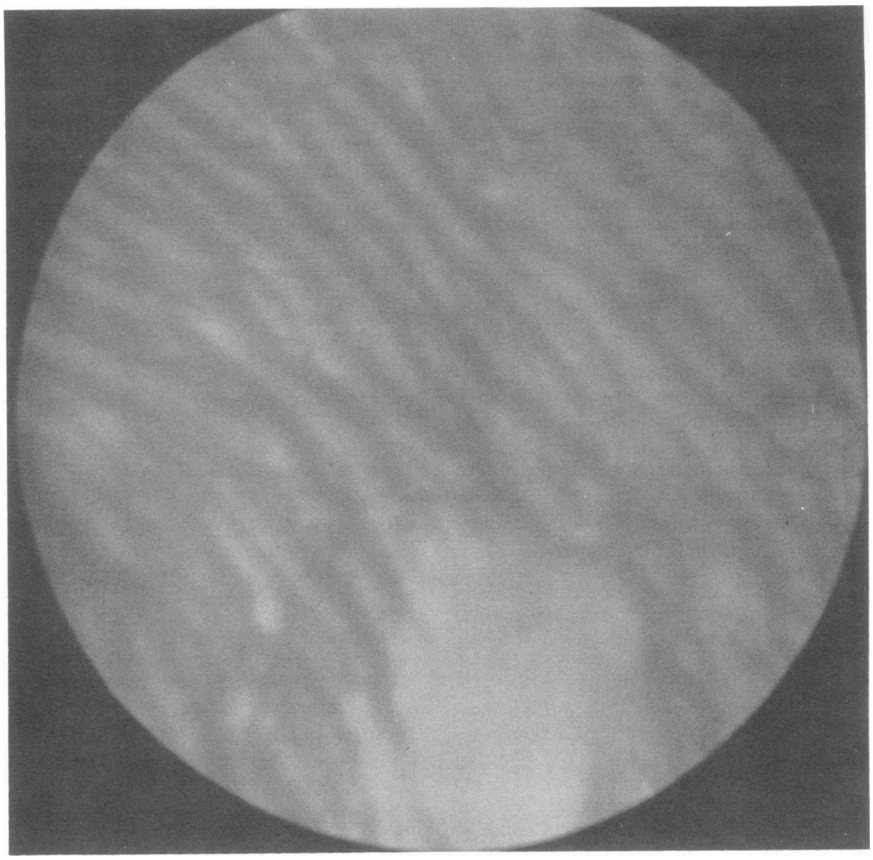

FIG. 2. *Typical lesion of experimental HCE in a normal rabbit. Magnification, ca. ×16.*

postmortem cultures are shown in Fig. 3. The administration of HN_2 increased the susceptibility of rabbits to disseminated candidiasis. The extent of infection in the kidneys of the severely immunocompromised rabbits was significantly increased as compared with controls ($P < 0.001$, Student's t test). Extensive infection was noted in the cultures of the chorioretinas of the most heavily immunocompromised rabbits, even though clinically detectable lesions were not seen.

DISCUSSION

The administration of quantities of HN_2 sufficient to suppress the bone marrow correlated inversely with the size and number of chorioretinal lesions seen in the rabbit model of HCE. The lesions that were seen in the most severely immunocompromised rabbits (group 3) were small and poorly formed. These data support our hypothesis that immunocompromised patients may not develop clinically detectable lesions of HCE with the same frequency as nonneutropenic patients. Furthermore, if the neutropenic, immunocompromised patient should develop a chorioretinal lesion due to *C. albicans*, the lesions may not be morphologically typical of HCE.

In our institution, we routinely examine the eyes of selected surgical patients who are at high risk for the development of disseminated candidiasis. The eyes are examined at least weekly with both direct and indirect ophthalmoscopy after having been fully dilated with mydriatics. Although physicians in other centers may not routinely employ these ophthalmoscopic techniques to detect HCE, the magnitude of the apparent difference in incidence of HCE in disseminated candidiasis in these two patient populations (i.e., the surgical population versus the immunocompromised patients) may be too great to be explained by procedural differences in the ocular examination.

The results of these experiments agree with previous studies from our laboratories in which the typical lesions of HCE were seen to be composed primarily of masses of inflammatory cells, with only a few organisms detectable in any lesion (2).

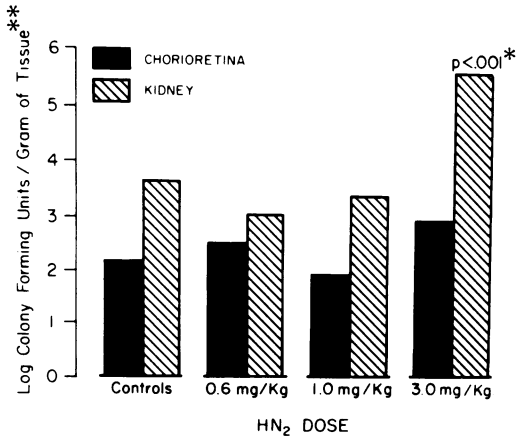

FIG. 3. *Extent of infection in chorioretina, vitreous, and kidneys in the four groups of rabbits. *, Student's t test comparing kidney culture results in 3.0-mg/kg HN_2-immunosuppressed rabbits to control kidney culture results. Chorioretina culture results were not statistically significant (Student's t test) from controls in any experimental group. **, Mean value for all rabbits sacrificed in the respective group. Numbers of rabbits surviving to the sacrifice day out of each group were as follows: control, 10/10, 100%; 0.6-mg/kg HN_2, 8/10, 80%; 1.0 mg/kg, 8/10, 80%, 3.0 mg/kg, 8/12, 66%. All rabbits sacrificed in each group were infected at necropsy.*

Administration of large doses of chemotherapeutic agents may suppress the bone marrow, prevent the accumulation of inflammatory cells in the chorioretina, and alter the ability of these cells to respond normally to an intraocular focus of infection. Clinical experience with severely immunocompromised patients has demonstrated that other focal infections (e.g., perirectal abscesses) may not be accompanied by a typical inflammatory response (4, 6–9).

Even though the frequency of detectable lesions in the severely immunocompromised, neutropenic rabbits was significantly diminished when compared to controls, we suggest that a careful ophthalmoscopic examination should still be a part of the evaluation of immunocompromised patients. Even the most severely immunocompromised neutropenic patient may develop a small lesion, the detection of which may confirm a suspected diagnosis of disseminated candidiasis.

From these studies, we conclude that the use of bone marrow-suppressing doses of HN_2 probably diminishes the inflammatory response in the eye and, for this reason, decreases the incidence of clinically detectable endophthalmitis in the rabbit model of disseminated candidiasis. For these reasons, ophthalmoscopic examination may not be a sensitive diagnostic modality for disseminated candidiasis in severely immunocompromised, neutropenic patients.

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