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A Mechanism for Liver Cell Injury in Viral Hepatitis: Effects of Hepatitis B Virus on Neutrophil Function *in Vitro* and in Children with Chronic Active Hepatitis

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Summary

Neutrophil function was studied by nitroblue tetrazolium (NBT) reduction, superoxide anion (O_2^-) production, chemotaxis, and bactericidal activity in 9 children with HBsAg-positive chronic active hepatitis (CAH). NBT reduction and O_2^- production were higher in resting neutrophils from the children with CAH than from the controls, but the production of O_2^- was depressed after stimulation with zymosan. No defect was observed in both random and direct locomotion, whereas a significant decrease was present in bactericidal activity. To evaluate the role of virus components, purified preparations of HBsAg and HBcAg were added to neutrophils from normal children. The incubation with such products induced *in vitro* the same modifications that were observed in the neutrophils from children with CAH. In addition, defects in phagocytosis and killing of *Candida albicans* as well as in chemotaxis were demonstrated. The production of O_2^- was reduced in the neutrophils, stimulated by zymosan and previously opsonized with HBsAg-positive serum.

Abbreviations

CAH, chronic active hepatitis
HBcAg, hepatitis B core antigen
HBsAg, hepatitis B surface antigen
HBV, hepatitis B virus
KRP, Krebs Ringer Phosphate
NBT, nitroblue tetrazolium
PMA, phorbolmyristate acetate
PMNs, polymorphonuclear neutrophils
SGOT, serum aspartate aminotransferase
SGPT, serum alanine aminotransferase

The mechanism by which HBV causes liver damage is unclear (35). Because individuals with persistent HBV infection and large numbers of virus particles in their blood frequently have little or no liver damage but patients with transient infections often have low titers of circulating HBV particles and severe liver damage, most investigators have concluded that viral replication, itself, is not the cause of liver cell death. Because patients with impaired immune functions (lepromatous leprosy, end stage renal disease, leukemia, etc.) are more likely to develop persistent infections than persons without impaired immune responses, several investigators have proposed that it is the immune response to HBV that is responsible for the damage to hepatocytes (8, 40). Recently, Dienstag *et al.* (14) and Levy and Chisari (35) reviewed various hypotheses that have been proposed to explain how lymphocytes or antibodies might be involved in the liver damage of hepatitis. They concluded that there was insufficient evidence to support any particular cellular or humoral mechanism.

An alternative possibility is that phagocyte cells, *i.e.*, PMNs and macrophages, may be involved in at least type B hepatitis. Alter-

ations in neutrophil function have been reported both in acute (37, 50) and in chronic hepatitis patients, as shown by reduced phagocytic activity (12, 56) and depressed chemotaxis (37). We previously observed that the bactericidal activity of neutrophils was reduced in children who were chronic carriers of HBsAg (57), most of whom had evidence of liver damage (13). We also demonstrated that the neutrophils of these patients had an activation of oxidative metabolism without phagocytosis, as shown by a higher proportion of stimulated PMNs in the NBT test (57). Similar observations had been reported by Hellum and Solberg (27) in patients with acute viral hepatitis (type unspecified).

Monocytes from patients with chronic hepatitis have shown high production and release of lysosomal enzymes, even without the stimulation of phagocytosis, suggesting that these cells had already been activated (20). Bradfield and Wells (10) indicated that Kupffer cells play an important role in the pathogenesis of hepatitis. Other investigators (17, 55) demonstrated in animal models that activation of macrophages may result in enhanced degranulation, release of lysosomal enzymes, and liver injury.

In this study we present data indicating that neutrophils (which represent a large compartment of circulating phagocytic cells) showed impaired function in children with chronic HBsAg-positive hepatitis. This alteration could be reproduced in *in vivo* experiments using PMNs from normal individuals exposed to purified HBV components, *i.e.*, HbsAg, HBcAg, or to HBsAg-positive serum. HBV components were able to promote a respiratory burst in resting PMNs with subsequent formation of the oxygen free radical O_2^-. We suggest that the formation of free radicals from chronically stimulated phagocytes plays a significant role in the pathogenesis of liver damage in patients with chronic HBsAg-positive hepatitis, and that this mechanism is probably important in the pathogenesis of chronic hepatitis.

Table 1. *Clinical, biochemical, and serologic data of nine children with chronic active hepatitis and as many controls*[1]

	Sex	Age at diagnosis (yr)	HBsAg	anti-HBs	SGOT[2]	SGPT[3]
Patients						
1	F	7	+	−	55	50
2	M	10	+	−	73	71
3	M	3	+	−	64	53
4	M	7	+	−	39	63
5	M	14	+	−	35	75
6	F	9	+	−	54	80
7	F	13	+	−	48	77
8	M	12	+	−	78	100
9	M	12	+	−	268	191
Controls						
1	F	7½	−	−	11	19
p 2	M	10	−	+	9	12
3	M	2½	−	−	21	13
4	M	6	−	−	7	9
5	M	14	−	−	14	15
6	F	9	−	−	19	11
7	F	13	−	+	10	21
8	M	12	−	−	18	12
9	M	11	−	−	14	17

[1] Abbreviations: HBsAg, hepatitis B surface antigen; HBs, antibody against HBsAg; SGOT, serum asparatate aminotransferase; and SGPT, serum alanine aminotransferase.

[2] IU/liter, normal range 7–40.

[3] IU/liter, normal range 5–25.

MATERIALS AND METHODS

This study included nine children, 3–14 years of age, with HBsAg-positive CAH diagnosed by the histologic criteria of Bianchi *et al.* (6, 7). All the patients presented high levels of amino transferases (SGPT and SGOT transaminases) for more than 1 year. HBsAg and HBsAb were detected by radioimmunologic methods (AUSTRIA and AUSAB, Abbott Lab., N. Chicago, IL). Controls consisted of as many sex- and age-matched children who were not infected with HBV. The clinical data of both groups are summarized in Table 1.

Separation of neutrophils. Venous blood was drawn into siliconized glass tubes containing 10 IU heparin per ml of blood. Mononuclear cells and platelets were separated from PMNs and red cells by centrifugation of Ficol-Isopaque ($\Delta = 1.007$ g/cm^3 at 20°C). Erythrocytes were removed by sedimentation for 45 min at 4°C in 6% dextran (molecular weight 500,000 Pharmacia Fine Chemicals, Inc., Piscataway, NJ) in saline, and those which still remained were lysed with 0.2% NaCl. Isotonicity was restored with an equal volume of 1.6% NaCl. PMNs were washed and resuspended in the buffer used in the particular experiment (usually KRP). This preparation did not interfere with the oxidative metabolism and function of cells. Cell viability was determined by the exclusion of 1% trypan blue dye.

Assay of locomotion. A modification of the method of Zigmond and Hirsch (60) was used as described by Wilkinson (58), which employs cellular suspensions containing 5×10^3 PMNs/ml in Sykes-Moore chambers or lucite blind well chemotactic chambers (Nucleopore, Pleasantown, CA) with a 5-μm millipore filter (Millipore Corp., Bedford, MA) to separate the upper from the lower compartment of the chambers.

Casein was used as the chemoattractant after solubilization in Hank's balanced salt solution by gently increasing the pH with NaOH to 12 and subsequent restoration to neutrality with 1.0 M NaH_2PO_4. A concentration of casein of 5 mg/ml was used in the attractant compartment. The chambers were incubated at 37°C for 45 min and the filters were fixed with ethanol and stained with hematoxylin. Triplicate chambers were run in each case, and the distance (μm) travelled by the leading front was measured at ×400 magnification. Five randomly chosen fields of cells were read for each filter and the results were pooled and the mean determined. Experiments performed using serum inactivated with 1 μg/ml of *E. coli* endotoxin produced analogous results.

In order to calculate the random migration, cells in Hank's balanced salt solution were allowed to migrate toward the medium without any chemoattractant.

In five experiments neutrophils from normal children were first incubated with 0.1 ml of HBsAg-positive serum or with purified HBsAg (250 μg/ml), or HBcAg (200 μg/ml) at 37°C for 30 min. Purified HBsAg, prepared by density gradient centrifugation (42) and a preparation of HBcAg from a liver of a HBsAg-positive chronic hepatitis patient (38), were denoted by Dr. I. Millman of the Institute for Cancer Research, Philadelphia, PA.

Bactericidal and candidacidal activity of neutrophils. Bactericidal activity of PMNs was assayed by the method of Quie *et al.* (46). *E. coli* 0 125 B were kindly supplied by the Sclavo Institute, Sienna, Italy. Bacterial cells, 2.5×10^6, and leukocytes, 2.5×10^6, were removed from patients and put in 1 ml of KRP containing 10% normal serum. Cells were incubated at 37°C. Samples were removed at 0, 15, 30, and 60 min. In four experiments bactericidal activity was tested in normal PMNs after 30 min' incubation with 40 μg of purified HBsAg, or 100 μl of HBsAg-positive serum. Controls were performed using the same neutrophils incubated with 100 μl of KRP or normal serum. After incubation cells were washed twice and bactericidal activity was assayed as reported above, also including a reading at 120 min. The number of viable *E. coli* was determined by colony count using the pourplate technique after 24 h incubation at 37°C, and was expressed as a % of the survival of the initial number of bacteria.

Candidacidal activity was evaluated by the method of Brune *et al.* (11) with minor modifications. Briefly, neutrophils from normal control children suspended in 100 μl KRP were allowed to adhere to slides and were incubated at 37°C for 30 min with 100 μl of KRP alone or containing 25 μg of HBsAg or 10 μg of HBsAg.

After incubation, slides were washed in KRP and exposed to 100 μl of 2×10^6 *Candida albicans* suspended in 25% fresh normal human serum. After 50 min incubation at 37°C, methylene blue (2×10^{-4} in Hank's solution) was added to stain dead phagocytized *Candida albicans* blue; living cells remained unstained. The following estimates were made: phagocytic index = % phagocytizing PMNs; avidity index = mean number of ingested *Candida albicans*/phagocytizing PMNs; candidacidal activity = % phagocytized *Candida albicans* which were killed.

Oxidative metabolism. Unstimulated and stimulated neutrophils from patients and controls were assayed by both the histochemical NBT test and superoxide anion production (O_2^-). The histochemical NBT test was done by the method of Park *et al.* (45) as modified by Gifford and Malawista (21). In addition, PMNs were stained with Samson dye, fixation with methanol was avoided and slides were read immediately. The % of NBT-positive neutrophils in normal children ranged from 0–12% (mean = 6.5). The stimulated NBT test was performed with antigen/antibody complexes from horse antiserum to human serum proteins or human serum. It showed that 89–97% of neutrophils were NBT-positive in controls. The production of superoxide anion was determined both in patients and controls by the reduction of cytochrome *c* according to the method of Bellavite *et al.* (5), which assays the release of O_2^- from leukocytes in whole blood as well as from purified preparations of PMNs. The assay mixture consisted of 1.0 ml of 2.3 mM cytochrome *c* in KRP; 1×10^6 PMNs in 0.2 ml of either whole blood or purified preparations. The mixture was brought to a final volume of 1.0 ml with KRP. Paired reactions were performed employing 50 μg of superoxide dismutase (Sigma Chemical Corp., St. Louis, MO). To stimulate the respiratory burst, zymosan (Sigma Ch. Corp., St. Louis, MO), phorbolmyristate-acetate (PMA) (Sigma Ch. Corp., St. Louis, MO), or purified preparations of HBsAg or HBcAg were used. Zymosan was prepared by boiling in saline for 15 min and then washed three times in the same medium. It was subsequently opsonized at 37°C for 30 min with 10% normal serum in KRP, and then washed and adjusted to a concentration of 10 mg/ml in KRP; 0.1-ml amounts were used in the test. In five experiments zymosan was opsonized with normal or HBsAg-positive serum to stimulate normal PMNs. PMA (0.2 mg/ml in dimethylsulfoxide) was used in the amount of 0.1 μg in the test. Purified preparations of HBsAg and HBcAg were used at a concentration of 250 and 100 μg/ml respectively; 10^6 normal neutrophils were preincubated with 0.1 ml of these preparations and washed twice in KRP before testing.

The viability of PMNs was evaluated after incubation in all the experiments, which included preincubation of these cells with purified HBsAg, HbcAg or HBsAg-positive serum.

RESULTS

Locomotion. Random migration did not show significant differences between children with HBsAg-positive CAH and the HBsAg-negative controls. There were also no statistically significant differences between children with CAH and the controls in the test for chemotaxis (Fig. 1). The effect of HBsAg-positive serum and of purified HBsAg and HBcAg on the chemotaxis of PMNs from normal children is shown in Figure 2. These cells presented a reduction of chemotaxis higher than 40% with respect to values obtained after incubation with normal serum; no differences were observed between the inhibition obtained with HBsAg, HBcAg or HBsAg-positive serum.

Bactericidal and Candidacidal activity. Killing of *E. coli* by neutrophils from children with CAH and from normal children was similar after 15 and 30 min incubation. Survival of *E. coli* was significantly greater after 60 min incubation with neutrophils from children with CAH than with cells from the controls ($P < 0.01$) (Fig. 3). As shown in Table 2, incubation with either purified HBsAg or HBsAg-positive serum resulted in a significant decrease in the bactericidal activity of normal neutrophils. Inhibition of this function was significantly greater with purified HBsAg than with HBsAg-positive serum and was more positive after 120 min incubation.

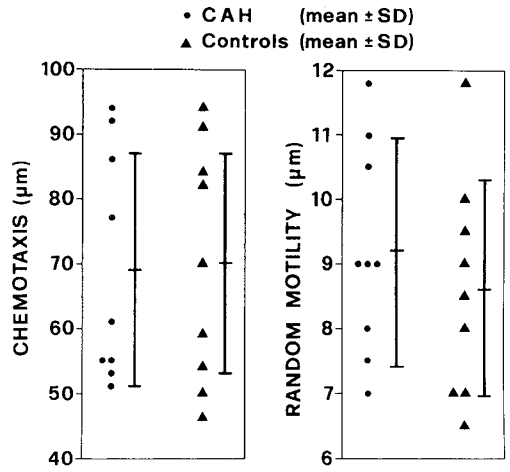

Fig. 1. Chemotaxis and random motility of neutrophils from children with chronic active hepatitis (CAH) and controls. The vertical bars represent ±1 S.D.

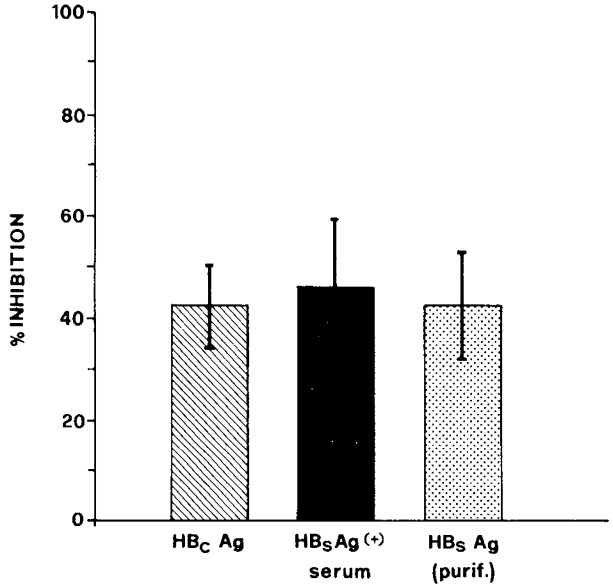

Fig. 2. % inhibition of chemotaxis in neutrophils from normal children preincubated with HBcAg, HBsAg-positive serum or HBsAg (purified preparation).

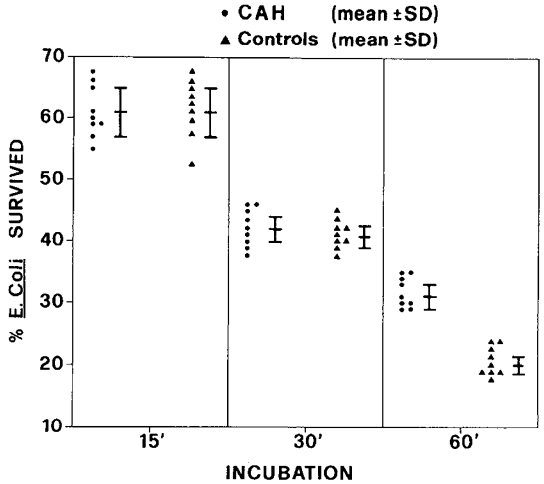

Fig. 3. Bactericidal activity of neutrophils from children with chronic active hepatitis (CAH) and controls. Data are expressed as % bacteria survived after 15, 30, and 60 min incubation.

Phagocytic index, avidity index, and candidacidal activity of normal PMNs incubated with HBsAg, HBcAg, and KRP are shown in Figure 4. HBcAg appeared to significantly ($P < 0.01$) inhibit the phagocytic index (Fig. 4, column *a*), the avidity index (Fig. 4, column *b*), as well as the candidacidal activity (Fig. 4, column *c*). HBsAg affected the avidity index (Fig. 4, column *b*) ($P < 0.01$) and the candidacidal activity (Fig. 4, column *c*) ($P < 0.01$), but not the phagocytic index.

Oxidative metabolism. NBT reduction was increased in all nine children with HBsAg-positive CAH when unstimulated PMNs were tested compared with normal children ($P < 0.01$) (Fig. 5, column *a*). Values in the stimulated NBT test obtained by using antigen/antibody complexes were similar in patients and controls (Fig. 5, column *b*).

The production of O_2^- showed significant differences between resting PMNs from children with CAH and from normal children ($P < 0.01$) (Fig. 6, column *a*). After stimulation with zymosan, PMNs from children with CAH showed reduced capacity to produce O_2^- compared with cells from normal children ($P < 0.01$) (Fig. 6, column *b*). On the contrary, stimulation with PMA did not reveal a significant difference between the two groups of children (Fig. 6, column *c*). In the experiments done with PMNs from normal children stimulated with zymosan opsonized with normal serum or with HBsAg-positive serum, the production of O_2^- was significantly different (51.1 ± 1.68 nmole *versus* 37.72 ± 2.73 nmole, $P < 0.01$). The effect of zymosan and PMA on the production of O_2^- by PMNs preincubated with normal serum or HBsAg-positive serum is shown in Table 3, whereas the effect of preincubation of neutrophils from normal children with purified preprations of HBsAg and HBcAg is illustrated in Table 4. As shown in these tables, preincubation of neutrophils with HBsAg-positive serum or with purified preparations of HBsAg and HBcAg resulted in a significant increase in O_2^- production when unstimulated cells were tested. This is in agreement with the observation that unstimulated neutrophils from children with HBsAg-positive CAH had a higher production of O_2^- and a higher % of NBT reducing cells, compared with neutrophils from normal children. On the other hand, phagocytosis of zymosan particles opsonized with either HBsAg-positive serum or normal serum in neutrophils preincubated with HBsAg or HBcAg induced a lower O_2^- production and decreased bactericidal activity. A normal production of O_2^- was obtained from neutrophils exposed to HBsAg and stimulated with PMA, under the same conditions as with zymosan. A significant decrease, however, was observed when neutrophils were preincubated with HBcAg.

Table 2. *Bactericidal activity of normal neutrophils incubated with normal or hepatitis B surface antigen (HBsAg)-positive sera or with HBsAg (mean ± S.D. of quadruplicate experiments)*

System	% Bacteria survived: Incubation = 60 min	% Bacteria survived: Incubation = 120 min	P[1]
(*a*) Normal neutrophils + normal serum	22.25 ± 2.21	7.84 ± 1.12	
(*b*) Normal neutrophils + HBsAg(+) serum	49.0 ± 1.82	16.63 ± 4.69	<0.01
(*c*) Normal neutrophils + HBsAg	64.75 ± 2.98	22.65 ± 4.95	<0.01

[1] *a* vs *b* and *c* after 60 and 120 min incubation.

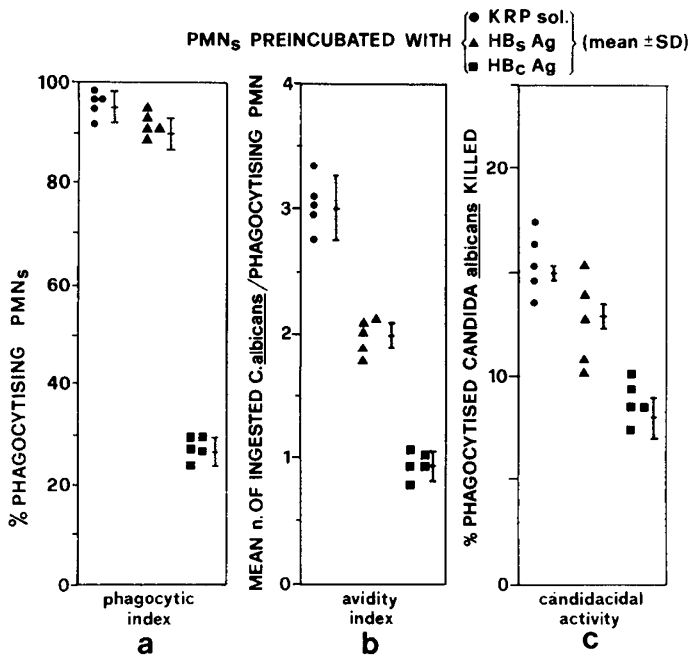

Fig. 4. Phagocytic index (*a*), avidity index (*b*), and candidacidal activity (*c*) of normal neutrophils after incubation with either purified HBsAg or HBcAg.

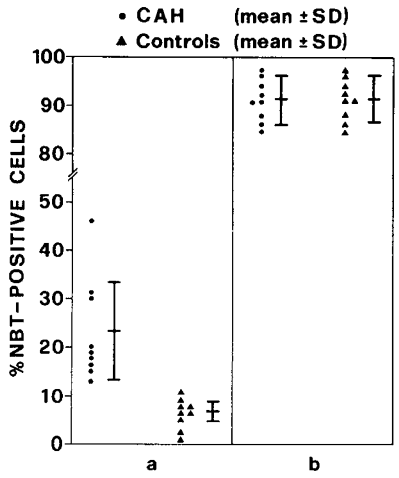

Fig. 5. Unstimulated (*a*) and stimulated (*b*) NBT test in neutrophils from children with chronic active hepatitis (CAH) and controls.

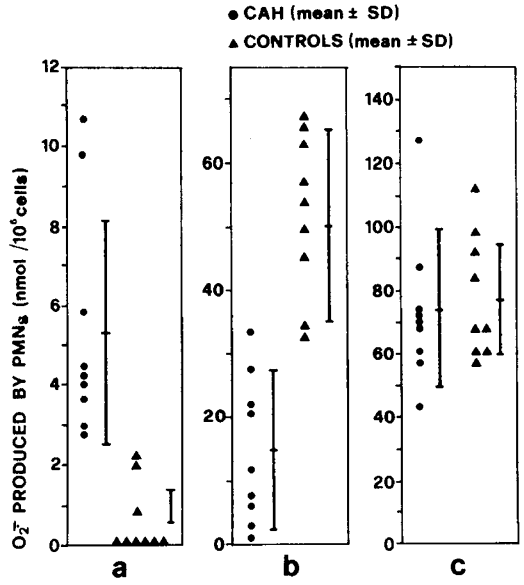

Fig. 6. Production of O_2^- by neutrophils from children with chronic active hepatitis (CAH) and controls, in resting state (*a*) and after stimulation with zymosan (*b*) and phorbolmyristate acetate (PMA) (*c*).

DISCUSSION

In this study we observed that children with HBsAg-positive CAH have alterations of neutrophil function consisting of: *(1)* increase in oxidative metabolism of resting cells as demonstrated by the increase of O_2^- generating activity and the increase in NBT reduction in unstimulated cells; *(2)* reduced O_2^- formation after stimulation of phagocytosis with activated zymosan; and *(3)* reduced capacity to kill ingested bacteria after 60 min incubation.

We tested the hypothesis that these functional modifications were related to the presence of HBV components. This was done by incubating normal neutrophils with purified HBsAg and HBcAg preparations before carrying out the functional assays. Alterations in function similar to those observed in neutrophils from HBsAg-positive children were induced *in vitro*. These included reduced phagocytosis and the killing of *Candida albicans* and of *E. coli*, an increase of oxidative metabolism in unstimulated neutrophils, and a decrease in O_2^- production after zymosan stimulation. One effect observed *in vitro* but not in neutrophils from children with CAH was a reduction of chemotaxis. This is similar to what has been observed with Herpes simplex by Kleinerman *et al.* (33), with measles by Anderson *et al.* (2), and with influenza virus by Ruutu *et al.* (48). Because no defect in chemotaxis was demonstrated in neutrophils from the affected children, some compensatory mechanism(s) may be operating *in vivo*.

The most interesting finding in this study was that HBsAg-positive sera, as well as HBV components, were able to induce a respiratory burst in neutrophils, as demonstrated by release of O_2^-. Conversely, the same neutrophils showed a marked reduction in O_2^- production when stimulated with opsonized zymosan; however, stimulation of neutrophil respiratory burst obtained with PMA after preincubation with HBsAg, under analogous experimental conditions, yielded an efficient response. PMA is able to induce functional modifications similar to those observed during phagocytosis (47) and may activate an oxidative mechanism different from the one activated by opsonized zymosan (39). A similar observation was reported by Winston *et al.* (59) in neutrophils exposed to cytomegalovirus. Initiation of the respiratory burst in neutrophils, similar to that induced by HBV, has recently been demonstrated for virus-antibody complexes of influenza virus (43) and syncytial virus (31). The production of toxic oxygen radicals might provide a defense mechanism against some viruses in the absence of specific antibodies. This could have harmful effects by impairing subsequent phagocytic function (34, 36). We do not know whether the increased level of superoxide stimulated by viruses reflects increased production or decreased detoxification by enzymes such as superoxide dismutase or catalase which are present in hepatocytes.

The activation of the respiratory burst in neutrophils is primarily related to antimicrobial defense (4), but it is also possible that oxygen radicals may be involved in mechanisms of inflammation and tissue damage (19). Oxygen radicals have been reported to induce peroxidation in lysosomal membranes (18), depolymerize hyaluronate (9), influence the growth and differentiation of myoblasts (41), activate platelet function (25) and cause endothelial (49) and lung damage (29, 53). Neutrophils stimulated by Con A produce free radicals that are cytotoxic to chicken erythrocytes (24), whereas stimulation by PMA induces formation of oxygen species within rat renal glomeruli, which may result in glomerular injury (52). Recently, it has been reported that the formation of oxygen free radicals interferes with lymphocyte function, leading to cell membrane damage (44). Further, it has been suggested that in rheumatoid arthritis, iron stored within synovial membranes catalyzes the production of hydroxylic radicals and subsequent lipidic peroxidation (9). High iron levels have also been found in HBsAg-positive patients (15), in β-thalassemia and in chronic active hepatitis (16). Iron might be responsible for the formation of radicals toxic for hepatocytes (23). The hypothesis can be advanced that macrophages activated through various stimuli may produce oxygen radicals that subsequently cause cellular damage. This mechanism may operate in liver tissue in the presence of iron overload or HBV or immune complexes. In acute and chronic hepatitis, immunoglobulins and immune complexes have been detected on the surface of hepatocytes (1, 3) where receptors for Fc and C_3 are present (28). As observed by Johnston and Lehmeyer (30), production of O_2^- occurs after neutrophil stimulation with immune complexes. Similar observations were reported by Goldstein *et al.* (22) in a system in which neutrophils had been stimulated with aggregated gammaglobulins and C_{5a}. Finally, immune complexes can alter phagocytic function (54).

From observations in the present study, we can speculate that the production of O_2^- and other products of activated oxygen by PMNs and probably by other phagocytic cells in the presence of HBV components may contribute to the liver injury in chronic hepatitis. Such damage might be mediated by monocytes, which are similar to neutrophils with regard to the production of O_2^- (32), and by Kupffer cells. Recently, it has been reported that cytochrome *b*-245, a major component of the oxidative system, is present in neutrophils, monocytes and macrophages (51). The above mentioned mechanisms differ from that proposed by Fer-

Table 3. *Release[1] of O_2^- by normal neutrophils preincubated with either normal serum or hepatitis B surface antigen (HBsAg)-positive serum (mean ± S.D. of quintuplicate experiments)*

	Neutrophils preincubated with		
Stimulus	Normal serum	HBsAg(+) serum	*P*
None	1.96 ± 0.92	10.32 ± 1.51	<0.01
Zymosan	61.20 ± 4.54	14.54 ± 1.42	<0.01
Phorbolmyristate acetate	115.24 ± 14.88	101.50 ± 4.15	NS

[1] Expressed as nmole/10^6 PMNs.

Table 4. *Release[1] of O_2^- by neutrophils from normal children preincubated with either hepatitis B surface antigen (HBsAg) or hepatitis B core antigen (HBcAg) purified preparations (mean ± S.D. of quadruplicate experiments)*

	Neutrophils preincubated with			
Stimulus	KRP solution	HBsAg	HBcAg	*P*
None	1.81^a ± 0.47	8.53^b ± 6.54	12.52^c ± 4.56	a *vs* c < 0.01 a *vs* b NS b *vs* c NS
Zymosan	58.93^a ± 5.89	19.97^b ± 6.41	17.02^c ± 9.16	a *vs* b < 0.01 a *vs* c < 0.01 b *vs* c NS
Phorbolmyristate acetate	77.30^a ± 5.42	68.95^b ± 5.79	63.73^c ± 2.05	a *vs* c < 0.01 a *vs* b NS b *vs* c NS

[1] Expressed as nmole/10^6 PMNs.

luga and Allison (17) and by Tanner *et al.* (55) in which the proteolytic enzymes released by activation of macrophages may promote hepatic injury. Although both mechanisms usually operate simultaneously, in viral hepatitis the formation of toxic oxygen radicals may have an earlier and more general effect because phagocytosis and degranulation are not required. Direct stimulation of the cell membrane of neutrophils or macrophages may be sufficient. The possibility remains that cells other than phagocytes may destroy hepatocytes through a mechanism analogous to the one we have suggested. In fact, it has recently been reported that human natural killer cells may induce cytolysis of specific target cells through the production of toxic free radicals (26).

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Neutrophil and T Lymphocyte Characteristics of Two Patients with Hyper-IgE Syndrome

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Summary

Immunologic parameters including quantitative and qualitative immunoglobulin studies, various T cell functions and neutrophil chemotaxis were evaluated in two patients with the Hyper-IgE syndrome. Both exhibited pruritic dermatitis in locations atypical for atopic dermatitis, marked elevations in serum IgE levels (to 40,000 IU/ml), recurrent staphylococcal abscesses, coarse facial features and variable chemotactic defects characteristic of this syndrome. Both patients responded favorably to courses of trimethoprim-sulfamethoxazole, particularly in helping control the cutaneous infections. We believe that this is a useful therapeutic alternative to anti-staphylococcal antibiotics and prophylactic treatment has permitted therapeutic response.

Serum IgG, IgG subclasses, IgM, and IgA were normal for age. Serum IgD was markedly deficient in one patient. Functional IgM was normal with positive isohemagglutinin titers. IgG poliovirus titers were present in both patients; however, tetanus titers were not detectable in either patient, despite repeated immunizations.

Despite normal E rosette numbers, subtle T cell abnormalities were noted with variable responses to both *in vivo* SK-SD, candida, and mumps skin tests and *in vitro* PHA-, Con A- tetanus-induced lymphocyte proliferation. Lymphocyte production of macrophage inhibitory factor and interferon and responsiveness in a mixed lymphocyte culture were normal in both patients. Considerable Con-A-induced suppressor cell activity was present in one patient, but diminished in the other.

***In vivo* chemotaxis determined by a Rebuck skin window, revealed a markedly delayed PMN migration in both patients during a time when both patients were clinically free of furunculosis or dermatitis. *In vitro* neutrophil chemotaxis was variable: maximally impaired in one patient during severe pneumonia and normalizing with clinical resolution; the low chemotactic activity of the second patient did not consistently correspond to the clinical state.**

Both patients demonstrated high serum histamine levels of 75–100 ng/ml (normal <1 ng/ml). The neutrophils of 8 of 11 normal adults showed significantly enhanced chemotaxis (26–52%) in the presence of 10^7 to 10^4 M histamine, and comparable enhancement with the same concentrations of the H_2 antagonist, cimetidine (17–38%). Both histamine and cimetidine consistently depressed chemotaxis in one patient. The other patient had enhanced chemotaxis in the presence of histamine and an inconsistent response to cimetidine. Histamine and cimetidine may affect neutrophils directly via H_2 receptors, indirectly via H_2 feedback inhibition of basophil histamine release or by non-H_2 mechanisms.

Abbreviations

CBC, complete blood count
CGD, chronic granulomatous disease
MIF, migration inhibition factor
MLC, mixed leukocyte culture
PHA, phytohemagglutin
PMN, polymorphonuclear leukocytes

Immunologic function, including quantitative and qualitative immunoglobulin studies, various T cell functions and neutrophil chemotaxis was evaluated in two patients with the Hyper-IgE syndrome (6, 9, 16). Both exhibited pruritic dermatitis, marked elevations in serum IgE levels (to 40,000 IU/ml), recurrent staphylococcal abscesses, coarse facial features, variable chemotactic defects and T cell defects characteristic of this syndrome (6, 9, 11, 12, 14, 16, 18, 23, 24, 27, 28, 53, 55). Additionally we report a favorable therapeutic response to trimethoprim-sulfamethoxazole, similar to that reported in CGD (33).

PATIENTS

Case 1. BB is a 20-year-old white female with a history of recurrent staphylococcal skin abscesses beginning in the first 2 weeks of life. As an infant she developed recurrent sinusitis and