Human granulocytic ehrlichiosis (HGE) is a tick-borne zoonosis caused by an obligate intracellular Ehrlichia species similar or identical to Ehrlichia equi and Ehrlichia phagocytophila (1, 3). Most infections are mild; however, on occasion severe complications, including opportunistic infections and fatalities, have been documented (14). E. phagocytophila infection of sheep and goats and E. equi infection in horses may be complicated by opportunistic infections as well (15). Except for serologic studies, immunologic function in humans with HGE has not been previously studied. Most infections by obligate intracellular bacteria require intact cell-mediated immunity for recovery from and protection against reinfection (5). To further characterize the human immune response to HGE and to ascertain if cytokine expression is associated with disease severity, we tested sera from patients during acute illness compared with those of patients during convalescence for the presence of the proinflammatory cytokines tumor necrosis factor alpha (TNF-α), interleukin 1β (IL-1β), gamma interferon (IFN-γ), IL-10, and IL-4 concentrations were elevated in acute-phase sera versus convalescent sera and normal subjects (P ≤ 0.013 and P ≤ 0.018, respectively). TNF-α, IL-1β, and IL-4 levels were not elevated. Cytokine levels in severely and mildly affected patients were not different. HGE leads to induction of IFN-γ-dominated cell-mediated immunity associated with clinical manifestations, recovery from infection, or both.

**RESULTS**

Patient demographic, clinical, and diagnostic evaluations. Fifteen patients with HGE were selected; nine had mild illness, and six had severe infections that required hospitalization (Table 1). Seven mildly affected patients and four severely affected patients came from the upper Midwest; the remaining two mildly and two severely ill patients came from southern New York State. Seven mildly ill patients and five severely ill patients had PCR evidence of HGE in their acute-phase blood. For PCR-negative patients, blood was obtained a median of 14 days after onset of illness versus 3.5 days in the PCR-positive group (P = 0.035).

Of the acute-phase sera of 15 patients with HGE, 11 were seronegative and 4 had HGE agent antibodies (geometric mean titer [GMT], 1,280) (overall GMT, 101). The GMT was 254 (range, <80 to ≥2,560) in the PCR-positive patients and 80 (range, <80 to 2,560) in the PCR-negative patients. The patients with antibodies in acute-phase sera were infected for a longer interval than antibody-negative patients (11 days versus 3 days; P = 0.036) before testing. Convalescent or second sera were obtained a median of 31 days after the onset of illness (range, 10 to 224 days). Eleven patients demonstrated seroconversions, two had stable high titers, one had a twofold increase in antibody titer, one PCR- and culture-positive indi-
were found between IL-10 or IL-1

elevations was of questionable significance. No differences
controls (not detected; $P$ 0.001). IFN-$\gamma$ concentrations in severely
affected patients were tested for both IFN-$\gamma$ and IL-10; $P$ 0.001). IFN-$\gamma$ concentrations were higher in acute-phase sera of HGE
patients (1,035 ± 66 pg/ml) than in convalescent sera (240
6 pg/ml) during convalescence (24 0.001). Similarly, serum concen-
trations in mildly affected patients (1,359 ± 410 versus
145 ± 66 pg/ml; $P < 0.001$). IFN-$\gamma$ levels in acute-phase serum

individual was seronegative in an assay of convalescent serum on
day 10, and one seronegative patient with morulae in the blood
during the acute phase had a convalescent titer of 80. The
convalescent serum GMT was 926, and no significant differ-
ence was observed in the titers whether or not the patients
were initially PCR positive ($P = 0.18$). Whole-blood samples
from five patients which were tested by PCR during the con-
valessent phase were all negative (Table 1).

Cytokine results. The results of cytokine ELISAs are shown in
Fig. 1. Sufficient serum for all cytokine tests was available from
two severely and three mildly affected patients and from
six healthy subjects. An additional 4 severely and 6 mildly
affected patients were tested for both IFN-$\gamma$ and IL-10, where-
as an additional 10 healthy subjects were tested for TNF-$\alpha$.
IFN-$\gamma$ concentrations were higher in acute-phase sera of HGE
patients (1,035 ± 235 [mean ± standard error of the mean] pg/
ml) than in convalescent sera (24 ± 22 pg/ml; $P < 0.001$) or
normal controls (not detected; $P < 0.013$). No difference was
observed between the IFN-$\gamma$ concentrations in severely and
mildly affected patients ($P = 0.60$). Similarly, serum concen-
trations of IL-10 were mildly elevated in patients during the
acute phase of HGE (118 ± 46 pg/ml) compared with those
during convalescence (24 ± 12; $P < 0.004$) and those in healthy
controls (not detected; $P < 0.019$). Acute-phase serum IL-10
concentrations were also elevated (15 ± 2 pg/ml) compared
with those of convalescent sera (8 ± 3 pg/ml; $P < 0.034$) and
controls (not detected; $P < 0.001$), but the magnitude of these
elevations was of questionable significance. No differences were
found between IL-10 or IL-18 concentrations in mildly and severely
affected patients with active HGE ($P = 0.32$ and 0.88, respectively).
In general, low concentrations or no IL-4 or
TNF-$\alpha$ were measured in most samples tested, and significant
differences were not found between any groups except when
acute-phase IL-4 levels were compared with those of healthy
controls ($P < 0.011$). A decreasing IFN-$\gamma$ concentration was
associated with the postonset interval ($r = -0.73$), and patients
without antibodies at presentation had higher levels of IFN-$\gamma$
in the serum than did seropositive patients (1,359 ± 410 versus
145 ± 66 pg/ml; $P < 0.001$). IFN-$\gamma$ levels in acute-phase serum

were similar ($P = 0.13$) regardless of whether ehrlichial DNA
was detected in the blood. Other cytokines were not detected
more frequently in patients with antibodies or ehrlichia DNA
present in acute-phase samples.

DISCUSSION

Recovery and protection from infections by obligate intra-
cellular bacteria often depend upon cell-mediated immune
responses resulting in production of IFN-$\gamma$ (5, 11). Here, evi-
dence is presented that patients with HGE develop not only
humoral immunity but also immune and proinflammatory cy-
tokine responses. Despite the various intervals of collection of
the sera tested, the height of some of these cytokine responses
provides an estimation of the relative concentrations during
acute phases of HGE.

HGE patients develop a mixed cytokine phenotype weighted
toward a TH1 response. The magnitude of the IFN-$\gamma$ response
in humans is similar to that observed in other rickettsial infec-
tions in humans (11). However, there are insufficient data to
link increased clinical severity with diminished TH1-type
immunity. While the overall importance of IFN-$\gamma$ in HGE is not
established, models of vasculotropic rickettsial infections show
that IFN-$\gamma$ in concert with TNF-$\alpha$ induces a nitric oxide-de-
pendent rickettsiadicidal mechanism that is critical for survival
and recovery (4). IFN-$\gamma$ also effects Ehrlichia risticii and Ehr-
lchiae chaffeensis destruction in macrophage cell lines by se-
questation of arginine and depletion of intracellular iron
stores, respectively (2, 12).

The source of these cytokines cannot be determined by the
present studies. However, HGE agent infection of dimethyl
sulfoxide-differentiated HL60 granulocytes induces chemok-
ines but not proinflammatory or immune cytokines (8). Thus,
infected cells are unlikely to be the primary source of IFN-$\gamma$
or IL-10 in HGE. Since the HGE agent rarely infects differenti-
ated macrophages (9), immune cytokines are probably gener-
ated after processing and presentation of ehrlichial antigens by
macrophages to T lymphocytes.

Strong evidence that IFN-$\gamma$ protects against infections by
obligate intracellular bacteria is provided by in vitro and in vivo
models, and IFN-$\gamma$ may protect against HGE as well. However,
protection has a consequence, as IFN-$\gamma$ is potentially damag-
ing to host cells (2, 4, 12). In HGE, tissue pathology and
clinical illness are greater than ehrlichial burden predicts (3,
14). Moreover, IFN-$\gamma$ secretion is associated with arthritis in
Borrelia burgdorferi-infected C3H/HeJ mice, and high levels are

![FIG. 1. Serum cytokine concentrations (mean ± standard error of the mean) in the acute (median, day 4 after onset) and convalescent (median, day 31 after onset) phases of HGE and in healthy adult control subjects.](image_url)
detected in the sera of patients with infection-associated hemophagocytic syndrome, a finding observed in humans and in animal models of HGE (6, 7, 14). In contrast, the anti-inflammatory effect of IL-10 may limit host-mediated tissue injury by down-regulating IFN-γ or other proinflammatory cytokines.

The presence of high acute-phase antibody titers in approximately 40% of HGE patients does not exclude an important role in recovery from infection or in immune-mediated disease (3). In vitro, antibody-E. chaffeensis complexes bind to Fc receptors of macrophages and elicit proinflammatory cytokine gene transcription and protein expression (10). However, the role of antibodies in cytokine-mediated inflammatory responses in vivo must be questioned, since the highest levels of IFN-γ were detected in patients prior to antibody responses, IFN-γ levels were negatively correlated with the interval after the onset of illness, and negligible quantities of TNF-α and IL-1β were detected. Passive transfusion of specific polyclonal antibody protects mice against challenge with the HGE agent (13). However, mice lack clinical signs of infection and immune reactions may not reflect those of infected humans (13). Our experiments with mice show a mixed TH response, with levels of IL-10 that may provide a degree of protection against the pathological effects of IFN-γ and other proinflammatory cytokines (11a).

High levels of IFN-γ and low levels of both IL-10 and IL-4 are produced with HGE, a phenotype most typical of a TH1 response, but no definite relationship between the presence or absence of cytokines and severity of illness has been demonstrated. Further studies will be required to confirm these findings and to elucidate the mechanisms of immunity for effective recovery from and protection against infection. Additional studies will be required to investigate whether immunopathologic processes are initiated and driven by the HGE agent or other E. phagocytophila group ehrlichiae that would suggest alternative strategies for the treatment and management of infected patients.

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