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MINI-REVIEW

Iron withholding: a defense against viral infections

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A variety of laboratory and clinical investigations during the past 15 years have observed that one of the dangers of excessive iron is its ability to favor animal viral infections. The metal is essential for host cell synthesis of virions and can also impair defense cell function and increase oxidative stress. In both animal models and humans, viral infections cause upregulation of the iron withholding defense system. Factors that suppress the system enhance viral progression; factors that strengthen the system augment host defense. Procedures designed to reinforce the system are being developed and tested; some of these may become useful adjuncts in prevention and management of viral diseases.

Keywords: iron, iron withholding defense, viral infections

Introduction

A fundamental attribute of virulence of bacterial, fungal, protozoan and neoplastic cells is their ability to obtain iron from their vertebrate hosts. In response, hosts attempt to withhold the growth-essential metal from the invading cells in several ways. These include (i) continuous stationing of potent iron-binding proteins at potential sites of invasion, (ii) lowering iron in body fluids and diseased tissues during invasion, and (iii) withdrawing non-heme iron from invaded host cells (Table 1).

Although viruses do not require iron, infected host cells need the metal to synthesize viral particles. Accordingly, it might be predicted that the iron-withholding defense system would be intensified during a viral invasion. As corollaries, conditions that impair iron withholding might enhance viral infections whereas factors that strengthen the defense system might suppress the infections. Evidence concerning these predictions is presented in this paper.

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Viral infections upregulate iron withholding

Effects of the invasion-associated iron-withholding processes include a reduction in serum iron and transferrin iron saturation, and an increase in serum ferritin. The altered values indicate that the host is responding to the invasion; as the patient recovers, the values promptly return to normal. Recent reports illustrate that these induced processes occur in viral infections. For example, in a set of 20 children with chicken pox and seven with mumps (Cemeroglu & Ozsoylu 1994), the alterations were observed during the clinical phase of the diseases. When the children had recovered, the values had returned to normal (Table 2).

In a similar study (Olivares *et al.* 1993), 120 healthy infants were immunized with attenuated measles virus or with a combination of measles, mumps and rubella viruses as models of mild viral infections. Values for serum iron, transferrin iron saturation and serum ferritin were measured at the time of inoculation and again at 9 and 14 days post-vaccination. As the mild infections developed, serum iron and transferrin iron saturation decreased significantly, whereas serum ferritin increased

Table 1. Selected aspects of the iron withholding defense system[a]

Constitutive components
Siderophilins
Transferrin in plasma, lymphatic fluid, cerebrospinal fluid
Lactoferrin in tears, milk, and secretions of respiratory, gastrointestinal and genital tracts
Ferritin within host cells
Processes induced at time of invasion
Suppression of assimilation of dietary iron[b]
Suppression of iron efflux from macrophages that have digested hemoglobin of effete erythrocytes[b]
Synthesis of additional ferritin to sequester retained iron[b]
Synthesis of nitric oxide (from L-arginine) to effect withdrawal of non-heme iron from invaded cells[c]

[a] Modified with permission from Table 1 of Weinberg (1995).
[b] Activated by interleukin-1 or -6 or by tumor necrosis factor-α.
[c] Activated by IFN-γ.

Table 2. Mean iron values of 20 children with chicken pox and seven children with mumps[a]

	Stage of disease		
	At presentation	21 days later	*P* value
Serum iron (μM)	5.2	15.2	<0.01
Transferrin iron saturation (%)	10.9	26.6	<0.01
Serum ferritin (ng ml^{-1})	59.6	35.6	<0.01

[a] Adapted from a portion of data in Table 1 of Cemeroglu & Ozsoylu (1994).
Median age of children: 6 years.
Values for hemoglobin, hematocrit and mean corpuscular volume remained unchanged during the course of the diseases.

significantly. Within 3 weeks after the mild infections had resolved, normal values had returned.

In the late stages of AIDS, humans become *hypo*ferremic and *hyper*ferritinemic, and they withhold large amounts of the metal in bone marrow, brain white matter, muscle and liver (Boelaert *et al.* 1996). These alterations apparently result from exposure of the patients to numerous onslaughts of opportunistic bacterial, fungal and protozoan pathogens (Blumberg *et al.* 1984, Boelaert *et al.* 1996). Presumably, the initial HIV infection that had developed within a few weeks of exposure to the virus would have resulted in a temporary alteration of the iron values; however, published studies on this matter are not yet available.

The hypoferremic response to viral infection has been demonstrated not only in humans but also in animal models. In mice infected with Japanese encephalitis virus, serum iron and transferrin iron saturation each were lowered by 60% at the peak of the clinical phase (Bharadwaj *et al.* 1991). At the same time, hepatic iron content was elevated by 170%. Values returned to normal when the mice had recovered.

In viral invasions, alteration of iron availability occurs not only at the tissue level but also at the intracellular level. Interferon (IFN)-γ induces synthesis of nitric oxide which forms nitrosyl–iron–sulfur complexes and thus inhibits enzymes that employ non-heme iron prosthetic groups (Weinberg 1992, Karupiah & Harris 1995). Targeted enzymes include ribonucleotide reductase, *cis*-aconitase and mitochondrial oxidoreductases. In a study on vaccinia, nitric oxide inhibited viral progeny production by blocking viral DNA synthesis, late gene expression and virus particle formation. The action of nitric oxide was reversed by exogenous ferrous sulfate plus L-cysteine (Karupiah & Harris 1995). Nitric oxide-mediated inhibition of viral replication occurred also with herpes simplex-1 virus. Host cells protected by nitric oxide included a human epithelial cell line as well as a murine primary uterine cell culture.

Viral infections are enhanced by impaired iron withholding

The iron-withholding defense system is impaired by iron loading via parenteral, inhaled or ingested routes of entry of the metal. In humans who become iron loaded by these routes of entry, increased risk of bacterial infection (Weinberg & Weinberg 1995) and of neoplasia (Weinberg 1996) have been extensively documented. Similar risks for viral infections have been reported after iron loading by transfusion, inhalation or ingestion.

For example, in five HIV patients, plasma p24 was measured immediately before and after packed red blood cell transfusion and then 1, 2 and 4 weeks later (Mudido *et al.* 1994). (The iron content of packed red blood cells is approximately 1 mg ml^{-1}.) No change in p24 level occurred immediately after transfusion. However, each patient experienced a rise in p24 at 1 or 2 weeks after transfusion; the rise

averaged 67% (25–125%) over baseline. In a study of 196 HIV-1-infected patients, the frequency of cytomegalovirus infections (considered by the authors to be reactivation of latent infection) was increased with increasing numbers of whole blood transfusions ($P < 0.01$) (Sloand *et al.* 1994).

Tobacco contains 440–1150 μg iron g^{-1} and cigarette paper 420 μg iron g^{-1}. Approximately 0.1% of the cigarette iron is contained in mainstream smoke. Thus a one pack per day smoker could inhale several micrograms of iron per day (Weinberg 1993). Not surprisingly, the iron burden of alveolar macrophages (AM) of smokers is two to four times greater than that of AM of non-smokers. Bronchoalveolar macrophages, obtained from 13 otherwise healthy smokers (mean of one pack per day) and 13 healthy non-smokers, were infected with a macrophage-tropic strain of HIV-1 and monitored for virus production (Abbud *et al.* 1995) for 12 days. Peak HIV-1 p24 antigen level in culture supernatants of cells of the smokers was 31 394 ± 8295 versus 7037 ± 2550 pg ml^{-1} in cell supernatants of the non-smokers ($P < 0.002$).

In persons infected with HIV, progression to AIDS required a mean of 8.17 months in 43 smokers and 14.50 months in 41 non-smokers ($P = 0.003$) (Nieman *et al.* 1993). In a different study of HIV-1 seropositive patients (Clarke *et al.* 1993), the virus was isolated from bronchoalveolar lavage cells in 31 of 43 (72%) cigarette smokers compared with 18 of 42 (43%) non-smokers ($P = 0.01$).

Persons with hereditary hemochromatosis (HH) comprise about 0.3–0.8% of Caucasian populations. Because of an apparent defect in expression of iron-regulated proteins in cells of the duodenal mucosa, the patients absorb from a normal diet 2–4 mg iron per day instead of the health-consistent quantity of 1–2 mg. The hazardous oversupply is sequestered in parenchymal cells of liver, heart and selected endocrine glands.

In a set of 183 male (45 ± 11.3 years) and 56 female (48 ± 12.4 years) HH patients, antibody to hepatitis B virus core was found in 13% of the males and 2.1% of the females (Deugnier *et al.* 1991). In an age- and sex-matched control group of blood donors, 3.7% of the males and 1.8% of the females were positive for the antibody. The difference between the result in the HH males as compared with the controls was significant ($P < 0.005$).

In African siderosis, persons who consume a diet high in bioavailable iron and who have a postulated (non-HH) genetic defect accumulate massive amounts of the metal in both hepatic parenchymal cells and in macrophages (Gangaidzo & Gordeuk 1995). In these persons, a strong correlation has been observed between hepatitis B virus surface antigen and iron content of their Kupffer cells and spleens (Senba *et al.* 1985).

In a set of 74 patients with porphyria cutanea tarda, 76% had antibody to hepatitis C virus as compared with 1% of 205 normal controls $P < 0.001$) (Fargion *et al.* 1992). In the patients, 'a high prevalence of mild to moderate iron overload' was noted. Regrettably, quantitative data on this matter were not provided.

Hepatic iron overload is not always a requisite for development of viral hepatitis. In a study of 28 patients with chronic viral hepatitis (six, B; 13, C; one, D; eight, unknown), liver iron content was elevated in only four (Di Bisceglı *et al.* 1992). An observed correlation of serum aspartate aminotransferase with serum ferritin suggested to the authors that the raised serum iron and ferritin generally seen in this disease could result from release of the metal and protein from hepatocellular stores during liver necrosis.

However, other studies of chronic carriers of hepatitis B virus have found that the elevation of serum iron in these patients can be independent of liver damage reflected by serum transaminase levels (Felton *et al.* 1979, Blumberg *et al.* 1981, Lustbader *et al.* 1983). Indeed, in a group of hemodialysis patients, a high serum ferritin, observed 2 months prior to appearance of hepatitis B surface antigen in the serum, was correlated with the likelihood that the infection would become persistent (Lustbader *et al.* 1983).

Moreover, in a series of 79 patients with chronic viral hepatitis (types B, C or NANBNC) treated with IFN-α, 45 achieved either a full or partial response (Van Thiel *et al.* 1994). The mean hepatic iron content of the responders was 638 ± 118 μg g^{-1} dry wt whereas that of the non-responders was 1156 ± 283 μg g^{-1} dry wt ($P < 0.05$). Similarly, in a group of 40 patients with chronic hepatitis C who were treated with IFN-α, 16 responded and 24 failed to respond (Piperno *et al.* 1993). Mean serum ferritin of responders were 126 (3–1121) ng ml^{-1}; of non-responders, 436 (105–2740) ng ml^{-1} ($P < 0.005$). Mean hepatic iron content of responders was 550 (150–1510) μg g^{-1}; of non-responders, 1620 (200–5910) μg g^{-1} ($P < 0.001$). Serum ferritin and hepatic iron were elevated in only 25 and 6% of responders, but in 79 and 58% of non-responders, respectively ($P = 0.0008$).

In a set of 39 chronic hepatitis C patients treated with IFN-α 2a (Arber *et al.* 1995), serum iron of the 13 responders was 18.5 ± 7.9 μM and transferrin iron

saturation was 30 ± 10%; of the 26 non-responders, 30.4 ± 7.7μM and 53 ± 12% ($P < 0.001$). In a different study of 15 chronic hepatitis C patients treated with IFN-α, complete responders had significantly less iron in Kupffer cells than did non-responders ($P = 0.02$) (Barton *et al.* 1993).

Viral infections are suppressed by strengthened iron withholding

A considerable array of natural, environmental, nutritional and medical methods are available for strengthening the iron-withholding defense system (Weinberg 1996). Among these, at least four have been tested in viral-infected animal models or humans. One type of method is that of augmenting nitric oxide synthesis. For example, L-arginine, the sole substrate for nitric oxide production, cannot be synthesized by birds but must be present in their diet. Chickens fed additional L-arginine to elevate their serum level 3-fold were inoculated with Rous sarcoma virus (Taylor *et al.* 1991). Control birds, fed a basal diet with sufficient L-arginine for normal growth, likewise were inoculated with the virus. The subsequent tumor load in the arginine-enriched birds was less than 70% of that of the controls.

In murine macrophages inoculated either with ectromelia virus, vaccinia virus, or herpes simplex-1 virus, *N*-methyl-L-arginine (a competitive inhibitor of L-arginine) reversed IFN-γ-induced restriction of replication of between 73 and 100% (Karupiah *et al.* 1993). The reversal was overcome by addition of excess L- but not D-arginine.

A second type of method for strengthening iron withholding is that of removal of excessive amounts of the metal by phlebotomy. In a set of 10 non-HH patients with chronic hepatitis C (Hayashi *et al.* 1994), periodic phlebotomy resulted in a mean reduction of serum alanine aminotransferase activity from 152 ± 49 to 55 ± 32 IU l^{-1}. In five of the 10 patients, the level became normal. The authors concluded that removal of even a slight, non-cytotoxic amount of excess iron is beneficial to patients with chronic hepatitis C.

In a similar study (Bacon *et al.* 1993), eight patients with chronic hepatitis C who had failed to respond to IFN-α were phlebotomized five to 10 times (mean 7.9). The procedure achieved a reduction of their elevated serum ferritin and transferrin iron saturation levels to normal and was accompanied by a reduction in serum alanine transaminase from 174 ± 97 to 101 ± 19 IU l^{-1} ($P < 0.05$). However, only a 50% reduction in serum hepatitis C RNA level was achieved by phlebotomy.

A third type of method that is being developed for strengthening iron withholding is that of employing iron chelating drugs. The bacterial trihydroxamate, desferrioxamine (DF), has been used for several decades in the treatment of transfusional iron or aluminium overload. More recently, this iron chelator has shown efficacy against selected eucaryotic and procaryotic pathogens when tested in *in vitro* as well as in animal and human models (Weinberg & Weinberg 1995, Boelaert *et al.* 1996). Additionally, a number of studies have evaluated the potential use of DF in viral infections.

In infected cultures of human foreskin fibroblasts, 15 μM DF achieved a 90% reduction in replication of four strains of human cytomegalovirus (Cinatl *et al.* 1994). The viability of the host cells remained unaffected by concentrations of DF as high as 60 μM. However, in this system, DF failed to inhibit replication of herpes simplex virus 1 or 2. In tests with human hepatoma cells, 15 μM DF likewise was not cytotoxic but achieved only a 50% reduction in replication of hepatitis B virions (Schwarz & Korba 1990).

Evaluations of the *in vitro* activity of DF against HIV also have given varied results. In one study (Lazdins *et al.* 1991), 30 μM DF caused a 90% reduction of HIV-1 replication but, in this system, cytotoxicity occurred. In another report (Baruchel *et al.* 1991), 18 μM DF reduced reverse transcriptase activity by an amount comparable to that attained by 26 μM zidovudine (AZT). However, in contrast to AZT which completely suppressed HIV-1 protein synthesis, DF reduced production of protein by only 63%. In a third account (Tabor *et al.* 1991), 30 μM DF suppressed p24 replication and substantially reduced detectable levels of *gag* and *env* genes without altering host cell viability.

In another study on HIV, two provirally infected cell lines (U1 promonocytoid and ACH-2 lymphocytic) were submitted to oxidative stress by hydrogen peroxide (Sappey *et al.* 1995). In this system, 5 μM DF resulted in significant decreased reverse transcriptase activity without cytotoxicity. The anti-retroviral activity of DF also was observed in HIV-1-infected peripheral blood mononuclear cells stimulated with interleukin-2 (Sappey *et al.* 1995).

Thirty one thalassemic patients who acquired HIV via blood transfusions were treated with a daily dose of DF of less than 40 mg kg^{-1} (Costagliola *et al.* 1994). Within 6.5 years after seroconversion, 35% had entered stage IV of AIDS. Thirty three

similar patients had received a daily dose of DF of greater than 40 mg kg^{-1}. Within 6.5 years after seroconversion, only 11% had entered stage IV of AIDS. The difference in the rate of progression to stage IV of the two groups was significant ($P < 0.002$). Only 13% of the first group and 9% of the second group had microbial infections; accordingly, the chelator probably functioned either to directly inhibit viral replication or indirectly to prevent oxidative stress (Costagliola *et al.* 1994).

A fourth type of method for strengthening iron-withholding defense is that of preventing the release of transferrin-bound iron that normally would occur within the host cell in the acid environment of the endosome or lysosome. The weak bases, chloroquine and hydroxychloroquine, accumulate at these sites and elevate local pH thus restricting iron release. For instance, in human macrophages, chloroquine-induced inhibition of *Histoplasma capsulatum* was reversed by iron nitriloacetate, an iron compound that is soluble at neutral and alkaline pH, but not by ferritransferrin, which releases iron only in an acidic environment (Newman *et al.* 1994).

In two retroviral systems (HIV-1 and avian REV-A), chloroquine caused significant size reduction of the cell- and virus-associated surface glycoproteins, gp 120 of HIV-1 and gp 90 of REV-A (Tsai *et al.* 1990). The majority of the HIV-1 virions released were non-infectious and the total virus yield was reduced. In a subsequent study (Sperber *et al.* 1993), hydroxychloroquine inhibited HIV-1 reverse transcriptase production without injury to the host cells. Concentrations of 10 and 1 μM resulted in a 50% reduction in enzyme yields from $CD4^+$ cells and macrophages, respectively. Recently, forty asymptomatic HIV-1-infected patients ($CD4^+$ counts of 200–500/mm^3) were randomly assigned to receive either 800 mg/day hydroxychloroquine or placebo for eight weeks (Sperber *et al.* 1995). The amount of recoverable HIV-1 RNA in plasma declined significantly in the treated group while it increased in the placebo group ($P = 0.022$).

Perspectives

The observations summarized herein suggest that, in animal and human hosts, excessive iron favors viral infections. Iron loading not only can enhance host cell production of viral nucleic acids and proteins but also can compromise immune defense mechanisms (Weinberg 1996), enhance oxidative stress (Boelaert *et al.* 1996) and suppress cytokine-inducible nitric oxide synthesis (Weiss *et al.* 1994). Among the numerous methods for strengthening iron withholding (Weinberg, 1996), the following might be especially relevant in prevention and management of viral infections: (i) substitute erythropoietin for blood transfusions whenever possible (Fischl *et al.* 1990), (ii) stop use of tobacco and (iii) add iron reduction therapy (phlebotomy; iron chelators) for patients who are receiving IFN-α or other antiviral agents (Caraceni *et al.* 1994, Boelaert *et al.* 1996). Furthermore, persons with underlying conditions that promote iron overload should be urged to maintain strong immunization schedules for both viral and bacterial infectious diseases.

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References

Abbud RA, Finegan CK, Guay LA, Rich EA. 1995 Enhanced production of human immunodeficiency virus type I by *in vitro* infected alveolar macrophages from otherwise healthy cigarette smokers. *J Infec Dis* **172**, 859–863.

Arber N, Moshkowitz M, Konikoff F, *et al.* 1995 Elevated serum iron predicts poor response to interferon treatment in patients with chronic HCV infection. *Dig Dis Sci* **40**, 2431–2433.

Bacon BR, Rebholz AE, Fried M, Di Bisceglı AM. 1993 Beneficial effect of iron reduction therapy in patients with chronic hepatitis C who failed to respond to interferon-α. *Hepatology* **18**, 150A.

Barton A, Cable E, Smith L, Banner B, Bonkovsky H. 1993 Distribution of iron in the liver predicts the response of chronic hepatitis C infection to interferon therapy. *Hepatology* **18**, 150A.

Baruchel S, Gao Q, Wainberg MA. 1991 Desferrioxamine and HIV. *Lancet* **337**, 1356.

Bharadwaj M, Khanna N, Mathur A, Chaturvedi UC. 1991 Effect of macrophage-derived factor on hypoferraemia induced by Japanese encephalitis virus in mice. *Clin Exp Immunol* **83**, 215–218.

Blumberg BS, Lustbader Ed, Whitford PL. 1981 Changes in serum iron levels due to infection with hepatitis B virus. *Proc Natl Acad Sci USA* **78**, 3222–3224.

Blumberg BS, Hann H-WL, Mildvan D, Mathur U, Lustbader E, London WT. 1984 Iron and iron binding proteins in persistent generalized lymphadenopathy and AIDS. *Lancet* **i**, 347.

Boelaert JR, Weinberg GA, Weinberg ED. 1996 Altered iron metabolism in HIV infection: mechanisms, possible consequences, and proposals for management. *Infect Agents Dis* **5**, 35–46

Caraceni P, Fagiuoli S, Van Thiel DH. 1994 Iron reduction therapy: simply camoflage, or a real weapon? *Am J Gastroenterol* **89**, 971–973.

Cemeroglu AP, Ozsoylu S. 1994 Haematologic consequences of viral infections including serum iron status. *Eur J Pediatr* **153**, 171–173.

Cinatl J Jr, Cinatl J, Rabenau H, Gumbel HO, Kornhuber B, Doerr HW. 1994 *In vitro* inhibition of human cytomegalovirus replication by desferrioxamine. *Antiviral Res* **25**, 73–77.

Clarke JR, Taylor IK, Fleming J, Nukuna A, Williamson JD, Mitchell DM. 1993 The epidemiology of HIV-1 infection of the lung in AIDS patients. *AIDS* **7**, 555–560.

Costagliola DG, de Montalembert M, Lefrer J-J, *et al.* 1994 Dose of desferrioxamine and evolution of HIV-1 infection in thalassemic patients. *Br J Haematol* **87**, 849–852.

Deugnier Y, Battistelli D, Jouanolle H, *et al.* 1991 Hepatitis B virus infection markers in genetic haemochromatosis. *J. Hepatol* **13**, 286–290.

Di Biscegli AM, Axiotis CA, Hoofnagle JH, Bacon BR. 1991 Measurements of iron status in patients with chronic hepatitis. *Gastroenterology* **102**, 2108–2113.

Fargion, S, Piperno A, Cappelini MD. 1992 Hepatitis C virus and porphryia cutanea tarda. *Hepatology* **16**, 1322–1326.

Felton C, Lustbader ED, Merten C, Blumberg BS. 1979 Serum iron levels and response to hepatitis B virus. *Proc Natl Acad Sci USA* **76**, 2438–2441.

Fischl M, Galfin JE, Levine JD, *et al.* 1990 Recombinant human erythropoietin for patients with AIDS treated with zidovudine. *New Engl J Med* **322**, 1488–1493.

Gangaidzo IT, Gordeuk VR. 1995 Hepatocellular carcinoma and African iron overload. *Gut* **37**, 727–730.

Hayashi I, Takikawa T, Nishimura N, Yano M, Isomura T, Sakamoto N. 1994 Improvement of serum aminotransferase levels after phlebotomy in patients with chronic active hepatitis C and excess hepatic iron. *Am J Gastroenterol* **89**, 986–988.

Karupiah G, Harris N. 1995 Inhibition of viral replication by nitric oxide and its reversal by ferrous sulfate and tricarboxylic acid metabolites. *J Exp Med* **181**, 2171–2179.

Karupiah G, Xie QW, Butler RM, Nathan C, Duarte C, MacMicking JD. 1993 Inhibition of viral replication by interferon-gamma-induced nitric oxide synthase. *Science* **261**, 1445–1448.

Lazdins JK, Alteri E, Klimkait T, *et al.* 1991 Lack of effect of desferrioxamine on *in vitro* HIV-1 replication. *Lancet* **338**, 1341–1342.

Lustbader ED, Hann H-WL, Blumberg BS. 1983 Serum ferritin as a predictor of host response to hepatitis B virus infection. *Science* **220**, 423–425.

Mudido P, George SD, Dorazio D, O'Brien WA, Lederman MM. 1994 Blood transfusion may increase HIV expression. Am Soc Microbiol. *Interscience Conf Antimicrob Agents Chemother* 130A.

Newman SL, Gootee L, Brunner G, Deepe GS Jr. 1994 Chloroquine induces human macrophage killing of *Histoplasma capsulatum* by limiting the availability of intracellular iron and is therapeutic in a murine model of histoplasmosis. *J Clin Invest* **93**, 1422–1429.

Nieman RB, Fleming J, Coker RJ, Harris JRW, Mitchell DM. 1993 The effect of cigarette smoking on the development of AIDS in HIV-1-seropositive individuals. *AIDS* **7**, 705–710.

Olivares M, Walter T, Llaguno S, Osorio M, Chadud P, Velozo L. 1993 Changes in the hemogram and in the laboratory parameters indicative of iron metabolism in mild viral infections. *Sangre-Barc* **38**, 211–216.

Piperno A, D'Alba R, Roffi L, Fargion S, Mancia G, Fiorelli G. 1993 Relation between α-interferon response and liver iron stores in chronic hepatitis C. *Hepatology* **18**, 250A.

Sappey C, Boelaert JR, Legrand-Poels S, Forceille C, Favier A, Piette J. 1995 Iron chelation decreases NF-κB and HIV type 1 activation due to oxidative stress. *AIDS Res Hum Retroviruses* **11**, 1049–1061.

Schwarz KB, Korba B. 1990 Desferrioxamine inhibits hepatitis B virus virion production by HepG2 cells. *Hepatology* **12**, 972A.

Senba M, Nakamura T, Hakura H. 1985 Statistical analysis of relationship between iron accumulation and hepatitis B surface antigen. *Am J Clin Pathol* **84**, 340–342.

Sloand E, Kumar P, Klein HG, Merritt S, Sacher R. 1994 Transfusion of blood components to persons infected with human immunodeficiency virus type 1: relationship to opportunistic infection. *Transfusion* **34**, 48–53.

Sperber K, Kalb TH, Stecher VJ, Banerjee R, Mayer L. 1993 Inhibition of human immunodeficiency virus type 1 replication by hydroxychloroquine in T cells and monocytes. *AIDS Res Hum Retroviruses* **9**, 91–98.

Sperber K, Louie M, Kraus T, Proner J, Sapira E, Lin S, Stecher V, Mayer L. 1995 Hydroxychloroquine treatment of patients with human immunodeficiency virus type 1. *Clin Therapeut* **17**, 622–625.

Tabor E, Epstein JS, Hewlett IK, Lee SF. 1991 Inhibition of desferrioxamine of *in vitro* replication of HIV-1. *Lancet* **337**, 795.

Taylor RL Jr, Austic RE, Dietert RR. 1991 Dietary arginine influences Rous sarcoma growth in a major histocompatability complex progressor genotype. *Proc Soc Exp Biol Med* **199**, 38–41.

Tsai W-P, Nara PL, Kung H-F, Oroszlan S. 1990 Inhibition of human immunodeficiency virus infectivity by chloroquine. *AIDS Res Hum Retroviruses* **6**, 481–489.

Van Thiel DH, Friedlander L, Fagiuoli S, Wright HI, Irish W, Gavaler JS. 1994 Response to interferon-α therapy is influenced by the iron content of the liver. *J Hepatol* **20**, 410–415.

Weinberg ED. 1992 Iron depletion: a defense against intracellular infection and neoplasia. *Life Sci* **50**, 1289–1297.

Weinberg ED, 1993 Association of iron with respiratory tract neoplasia. *J Trace Element Exp Med* **6**, 117–123.

Weinberg ED, 1995 Acquisition of iron and other nutrients *in vivo*. In Roth JA, Bolin CA, Brogden KA, Minion FC, Wannemuehler MJ, eds. *Virulence Mechanisms of Bacterial Pathogens*. Washington, DC: American Society for Microbiology; 79–94.

Weinberg ED, 1996 The role of iron in cancer. *Eur J Cancer Prev* **5**, 19–36

Weinberg ED, Weinberg GA, 1995 The role of iron in infection. *Curr Opin Infect Dis* **8**, 164–169.

Weiss G, Werner-Felmayer G, Werner ER, Grunewald K, Wachter H, Hentze MW, 1994 Iron regulates nitric oxide synthase activity by controlling nuclear transcription. *J Exp Med* **180**, 969–976.