Increased Release of Ferritin and Iron by Iron-loaded Alveolar Macrophages in Cigarette Smokers

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The lower respiratory tract of cigarette smokers contains an increased amount of iron that is predominantly sequestered within alveolar macrophages (AM), but is also present in alveolar epithelial fluid. Extracellular ferritin-bound iron could potentially be released by reductants present in cigarette smoke and catalyze generation of highly reactive hydroxyl radicals capable of causing oxidant injury. To determine whether AM are a source of alveolar extracellular ferritin and iron, we assessed *in vitro* release of iron, ferritin, and transferrin by AM recovered by bronchoalveolar lavage (BAL) of 27 healthy subjects including nine nonsmokers (NS), nine light smokers (LS), and nine heavy smokers (HS). Release of iron *in vitro* over 20 h was increased in AM recovered from LS (2.24 ± 0.21 nmol/10^6 AM/20 h, $p < 0.001$) and HS (3.11 ± 0.32 nmol/10^6 AM, $p < 0.001$) compared with NS (1.28 ± 0.08 nmol/10^6 AM). Release of ferritin *in vitro* over 20 h was also increased in AM recovered from LS (71 ± 24 ng/10^6 AM, $p < 0.05$) and HS (176 ± 35 ng/10^6, $p < 0.001$) compared with NS (18 ± 3 ng/10^6 AM). AM recovered from 12 smokers (8 HS, 4 LS) contained greater than 10 nmol of iron per 10^6 cells. These iron-loaded AM released a greater percentage of cell ferritin stores *in vitro* over 4 h (8.4% ± 1.1, $p < 0.01$) than did AM from NS (3.2% ± 0.6). Release of lactate dehydrogenase (LDH) over 4 h was substantially less (2.9% ± 0.3, $p < 0.001$) than ferritin release. Ferritin release by AM *in vitro* over 20 h correlated with concentrations of ferritin present in BAL fluid ($r = 0.80$, $p < 0.001$) for all subjects. Transferrin release *in vitro* by AM from each group was similar. These data suggest that high concentrations of extracellular ferritin and iron in alveolar structures of some cigarette smokers may be derived, in part, from release of ferritin and iron by iron-loaded AM. **Wesselius LJ, Nelson ME, Skikne BS. Increased release of ferritin and iron by iron-loaded alveolar macrophages in cigarette smokers. Am J Respir Crit Care Med 1994;150:690–5.**

Iron is an important catalyst of oxidant-induced injury because of its role in generating highly reactive hydroxyl radicals (OH) from less reactive superoxide anion and hydrogen peroxide via the Fenton reaction (1).

$$Fe^{3+} + O_2^- \rightleftarrows Fe^{2+} + O_2$$

$$Fe^{2+} + H_2O_2 \rightleftarrows Fe^{3+} + OH + OH$$

Prior studies indicate that the lower respiratory tract burden of iron is increased in cigarette smokers (2–5). The greatest amount of this iron burden is sequestered within alveolar macrophages (AM) in ferritin and hemosiderin, which effectively inhibits local iron-catalyzed hydroxyl radical formation (6). Increased amounts of extracellular ferritin and iron are also present, however, in alveolar structures of smokers as indicated by increased recovery of iron and ferritin in bronchoalveolar lavage (BAL) fluid. Recent studies indicate that cigarette smoke contains reducing agents that are capable of releasing iron from ferritin, making extracellular ferritin a potential source of iron to catalyze generation of toxic hydroxyl radicals (7). The generation of highly reactive oxidants in the lungs of cigarette smokers may have a role in the pathogenesis of emphysema by impairing antiprotease function, directly attacking lung matrix proteins or by inactivating enzymes involved in elastin synthesis and lung repair (8–10). Experimental support that oxidants may play a role in the pathogenesis of emphysema is provided by studies demonstrating that exposure to the oxidant stress associated with hyperoxia (60%) enhances the development of emphysema in elastase-treated hamsters (11).

The source of the increased extracellular ferritin and iron in alveolar structures of smokers is unclear. AM are known to play an important role in taking up iron and sequestering it within intracellular ferritin (6). Prior studies also indicate that macrophage populations may release iron bound to ferritin and/or transferrin following iron loading (12–14). We hypothesized that under conditions of smoking-induced iron loading, AM in smokers might release increased amounts of ferritin and iron. To examine this possibility we quantitated BAL fluid content of iron and iron-binding proteins, as well as *in vitro* release of iron and iron-binding proteins, by AM recovered from cigarette smokers and nonsmokers.

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METHODS

Subjects

Studies were performed in 27 healthy volunteers: nine nonsmokers (NS), nine light smokers (LS) (≤ 1 pack per day), and nine heavy smokers (HS) (> 1 pack per day). All subjects denied any history of significant pulmo-

nary symptoms, although three of the heavy smokers and two of the light smokers described an intermittent nonproductive cough. The ages of study subjects were: NS (34.2 ± 3.3 mean ± SD), LS (40.4 ± 3.9), and HS (37.2 ± 4.6) (differences NS, $p > 0.05$). Total pack-years of smoking were significantly greater in HS (63 ± 25 pack-years) compared with LS (25 ± 13 pack-years). No subject was taking any medications. The experimental protocol was approved by the Human Subjects Review Committee of the Kansas City Department of Veterans Affairs Medical Center and all subjects gave written informed consent.

BAL

Topical anesthesia with tetracaine was administered in the oropharynx and larynx. Subjects received intravenous midazolam and/or meperidine, and oxygen was administered during the procedure by nasal cannula. The bronchoscope was passed transorally and wedged in a subsegment of the right middle lobe. BAL was performed by infusing 250 ml saline solution (0.9%) in 50-ml aliquots. Fluid recovered from the second and subsequent saline infusions was pooled for subsequent analysis as an alveolar sample. Cells were recovered from BAL fluid by centrifugation (200 × *g* for 10 min) and total cell counts were determined with a hemacytometer. BAL fluids were stored at −20° C for later analysis.

Cell Culture

Cells recovered by BAL were suspended in RPMI 1640 containing 10% fetal calf serum (FCS), 100 μg streptomycin, and 100 U penicillin. An aliquot of cells containing 1×10^6 AM was added to 35-mm culture plates and then incubated at 37° C for 1 h in 5% CO_2. At that time nonadherent cells were removed by gentle washing with media. After removal of nonadherent cells, either fresh media was added for further cell culture or adherent cells were recovered by gentle scraping with a rubber policeman. Cells were incubated at 37° C in 5% CO_2 for either an additional 4 h or 20 h at which time media was removed and centrifuged to remove any detached cells. In separate studies we determined that detachment of AM was somewhat greater for smokers (3.0 ± 1.1% at 4 h, 6.9 ± 1.8% at 20 h) than for nonsmokers (1.2 ± 0.4% at 4 h, 2.1 ± 0.8% at 20 h). Cells and culture media were subsequently analyzed for iron and ferritin content. In a subset of AM cultures from each group, lactate dehydrogenase (LDH) activity in cell fraction and in supernate was also determined.

Iron and Ferritin Content of Alveolar Macrophages, BAL Fluid, and Alveolar Macrophage Conditioned Media

Recovered macrophages were suspended at a known concentration of 0.9% saline and sonicated. The concentration of iron was determined using 25-μL samples in duplicate in a controlled coulometry method (Ferrochem II; Environmental Science Associates, Bedford, MA) as previously described (15). Working standards for iron were prepared from certified ferric chloride suitable for standardization with atomic absorption spectrometry (Fisher Scientific, Fairlawn, NJ). This method has a sensitivity of 10 ng/ml. Aliquots of unconcentrated BAL fluid and AM conditioned media (20 h) were similarly assayed for iron content. The iron content of media used in macrophage cultures was also determined.

The ferritin content of AM and unconcentrated BAL fluid were determined using a solid-phase, two-side immunoradiometric assay using antibodies to L-type ferritin (Hybritech, San Diego, CA). This assay has a sensitivity of 0.7 ng/ml of ferritin. H-type ferritin was not assayed because prior studies demonstrated that concentrations of H ferritin in alveolar macrophage and BAL fluid are much lower than L ferritin and are increased only slightly in cigarette smokers (5). Conditioned media were recovered after either 4 or 20 h of macrophage culture, centrifuged (200 × *g* for 10 min) to remove any detached cells, and the supernate stored at −20° C.

Binding of Ferritin to Concanavalin A

The percentage of ferritin present in BAL fluid, AM supernates (20 h), and in AM lysates binding to concanavalin A was determined using a modification of the method described by Worwood and coworkers (16). In brief, Con A–Sepharose 4B (Pharmacia, Piscataway, NJ) was washed three times in assay buffer (0.05 M barbitone sodium; 0.1 M NaCl, 0.02% NaN_3, and 5 g/L bovine serum albumin adjusted to pH 8.0) using 10 times the packed volume. Conditioned media or BAL fluid (0.2 ml) was mixed using a roller mixer at room temperature for 1 h with 0.5 ml Con A–Sepharose 4B suspension and 0.3 ml assay buffer. After centrifugation (1,000 × *g* for 15 min) the supernatant was removed and ferritin concentrations present in supernate were determined as described previously.

Measurement of Transferrin Content of BAL Fluid and Alveolar Macrophage Conditioned Media

Aliquots of unconcentrated BAL fluid were assayed by ELISA to determine transferrin content using a combination of a polyclonal antibody and a monoclonal antibody as previously described (17). This assay has a sensitivity of 10 μg/L. Conditioned media recovered after 20 h of AM culture was similarly assayed for concentrations of transferrin. There was no cross-reactivity using this assay between human transferrin and bovine transferrin.

Total Protein and LDH Activity

The total protein content of BAL fluid was determined using the method of Bradford (18). LDH assays of AM supernates (20 h) were performed using the method of Elliott and Wilkinson with sodium pyruvate as substrate at 20° C (19). Results for culture supernate LDH are reported as a percentage of total LDH present in the sonicated cell layer.

Statistics

Values are reported as mean ± standard error (SE). Statistical analysis was performed using analysis of variance. For ferritin, statistics were performed using logarithms of original values.

RESULTS

Bronchoalveolar Lavage and Macrophage Recovery

BAL fluid recovery and total numbers of macrophages recovered from subjects in each group are provided in Table 1. AM recovery was significantly increased in both groups of smokers compared with nonsmokers. Protein concentrations present in BAL fluid were not significantly different between groups.

BAL Fluid Concentrations of Iron, Ferritin, and Transferrin

The iron content of BAL fluid recovered from NS was less than the lower limit of detection using our methods (10 ng/ml) in all nine subjects (Table 2). In LS, iron content of BAL fluid was greater than 10 ng/ml in four of nine subjects (range 10 to 20 ng/ml) with a mean iron content in these four of 12.5 ± 2.1 ng/ml. In HS, iron content of BAL fluid was greater than 10 ng/ml in all nine subjects (range 10 to 110 ng/ml) and the mean iron content was 49.7 ± 5.0 ng/ml.

The ferritin content of BAL fluid was significantly increased in both groups of smokers compared with nonsmokers whether expressed as ng/ml or as ng/mg total protein (Table 2). Comparing values expressed as ng ferritin/ml, BAL fluid from LS contained 7.7 times as much ferritin as BAL fluid from NS, and BAL fluid from HS contained 31.3 times as much ferritin. If values are expressed as ng ferritin/mg total protein, BAL fluid from LS contained 9.0 times as much ferritin as BAL fluid from NS, and BAL fluid

TABLE 1

BAL FLUID AND MACROPHAGE RECOVERY*

Subjects†	Fluid Recovery‡ (%)	Alveolar Macrophage Recovery (× 10^6)	BAL Fluid Protein (*mg/ml*)
Nonsmokers	72 ± 2	12.1 ± 1.6	0.183 ± 0.023
Light smokers	70 ± 2	41.3 ± 7.4§	0.214 ± 0.023
Heavy smokers	62 ± 3	91.2 ± 20.0§	0.254 ± 0.027

* Values are expressed as mean ± SE.
† n = 9 for each group.
‡ Percentage of infused fluid recovered.
§ $p < 0.001$ compared with nonsmokers.

TABLE 2

BAL FLUID CONTENT OF IRON, FERRITIN, AND TRANSFERRIN

	Iron Detected (> 10 ng/ml)	Ferritin (ng/ml)	Ferritin (ng/mgTP)	Ferritin (% binding to Con A)	Transferrin (μg/ml)
Nonsmokers	0/9 ND	9.9 ± 3.1	55 ± 15	ND	2.78 ± 0.68
Light smokers	4/9 mean 12.5 ± 2.1	76.9 ± 33.9*	497 ± 280*	31.9 ± 8.5	2.82 ± 0.29
Heavy smokers	9/9 mean 49.7 ± 5.0†	313.4 ± 88.1‡	1597 ± 416‡	52.2 ± 2.5	2.43 ± 0.31

Definition of abbreviations: BAL = bronchoalveolar lavage; TP = total protein; Con A = concanavalin A; ND = not detected.
* $p < 0.005$ compared with nonsmokers.
† $p < 0.001$ compared with light smokers.
‡ $p < 0.001$ compared with nonsmokers.

TABLE 3

AM RELEASE OF IRON, FERRITIN, AND TRANSFERRIN OVER 20 H

	Iron Content nmol/10^6 AM	Iron Released nmol/10^6 AM (% total AM iron)	Ferritin Content ng/10^6 AM	Ferritin Released ng/10^6 AM (% total AM ferritin)	Transferrin Release ng/10^6 AM
Nonsmokers	5.3 ± 0.4	1.28 ± 0.08 (25.2 ± 2.5%)	203 ± 2.5	18 ± 3.0 (9.2 ± 1.2)	58.1 ± 8.7
Light smokers	9.7 ± 1.8*	2.24 ± 0.21* (27.6 ± 3.0)	538 ± 135†	71.0 ± 24.3† (12.9 ± 2.6)	45.5 ± 5.9
Heavy smokers	23.2 ± 7.2*	3.11 ± 0.32* (18.6 ± 2.3)	1176 ± 214*	175.6 ± 34.8* (15.4 ± 2.4)	34.6 ± 6.8

* $p < 0.001$ compared with nonsmokers.
† $p < 0.05$ compared with nonsmokers.

from HS contained 29.0 times as much ferritin. In contrast to iron and ferritin, the transferrin content of BAL fluid was similar in NS, LS, and HS (Table 2).

Alveolar Macrophage Content of Iron and Ferritin

As noted in previous studies, the concentrations of iron and ferritin present in AM recovered from LS and HS were increased compared with concentrations present in AM recovered from NS (Table 3). In LS, AM iron content was increased 1.8 times and ferritin content was increased 2.7 times values in NS. In HS, AM iron content was increased 4.1 times and ferritin content was increased 5.8 times values present in NS.

Release of Iron, Ferritin, and Transferrin by Alveolar Macrophages *In Vitro*

Alveolar macrophages recovered from both groups of smokers released increased amounts of iron *in vitro* compared with NS over 20 h (Table 3). Alveolar macrophages from LS released 1.8 times as much iron and AM from HS released 2.4 times as much iron over 20 h as AM from NS. Whereas AM from NS and LS released a similar percentage of total iron stores (25.2 ± 2.5% and 27.6 ± 3.0%), AM from HS released less of total cell iron stores (18.6 ± 2.3%) over 20 h. This finding may be due to greater AM content of hemosiderin compared with ferritin in HS. Iron present in hemosiderin may not be available for cell release to the same extent as iron in cytosolic ferritin.

Alveolar macrophages from both groups of smokers released increased amounts of ferritin compared with that of NS after 4 h and after 20 h of culture (Figure 1). The increase in ferritin release by AM from smokers over 20 h was greater than the increase in release of iron. Alveolar macrophages from LS released 4.2 times as much ferritin as AM from NS, and AM from HS released 9.8 times as much ferritin over 20 h. In addition to differences in the absolute amount of ferritin release by smokers' AM, there were differences in the relative amount of ferritin released expressed as a percentage of total AM ferritin content both at 4 h and at 20 h of culture. There appeared to be a relationship between AM iron content and cell release of ferritin. AM with iron content greater than 10 nmol/10^6 AM (8 HS, 4 LS) released significantly more of total cell ferritin stores than did AM from NS or AM from the six

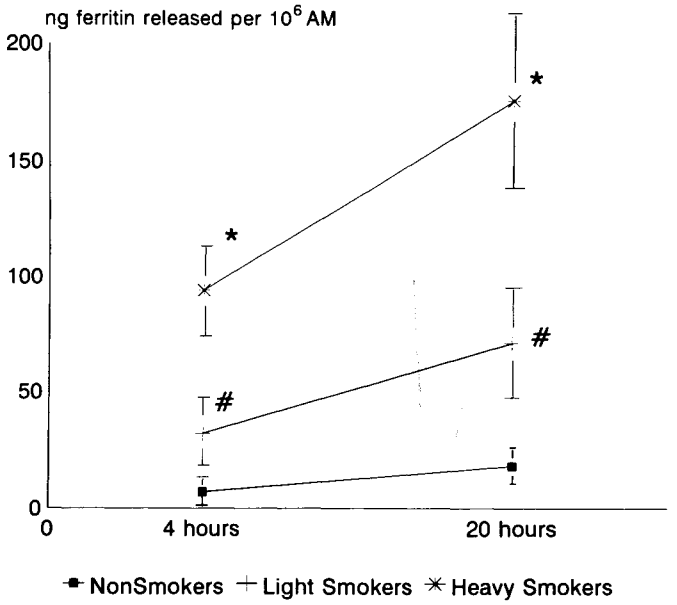

Figure 1. Time course of *in vitro* release by ferritin by AM recovered from each group of subjects. Values are mean ± SE (n = 9 in each group). #$p < 0.05$, *$p < 0.001$ compared with nonsmokers.

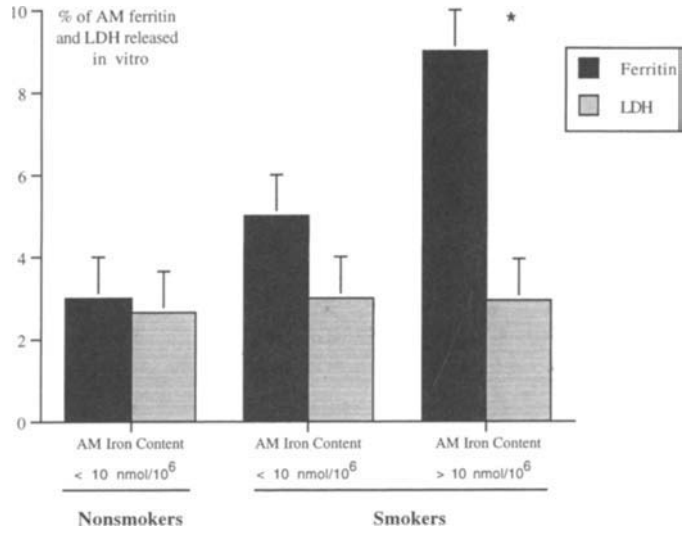

Figure 2. Percentage of total AM ferritin and lactate dehydrogenase (LDH) content released *in vitro* during 4 h of incubation for AM recovered from nonsmokers (n = 9), AM from smokers with iron content less than 10 nmol/10⁶ (n = 6, 5 LS, 1 HS), and AM from smokers with iron content greater than 10 nmol/10⁶ (n = 12, 4 LS, 8 HS). Values are mean ± SE. *Differences in percentage of ferritin released compared with NS, $p < 0.01$. Difference in percent release of ferritin and release of LDH, $p < 0.001$.

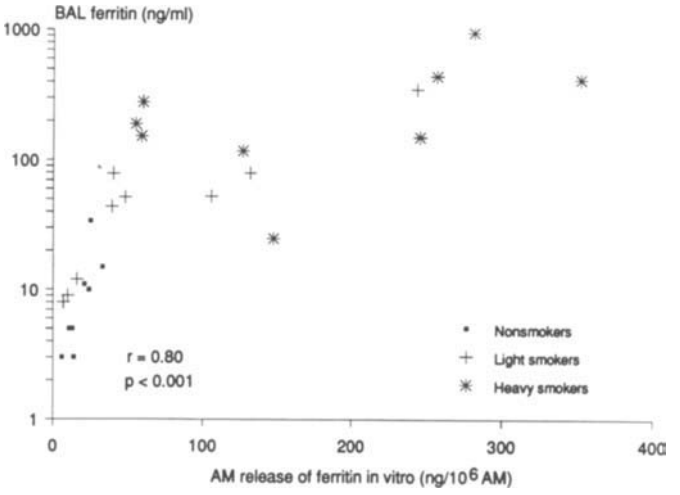

Figure 3. Correlation between concentrations of ferritin present in BAL fluid and amounts of ferritin released by AM *in vitro* during 20 h of culture.

smokers with AM iron content less than 10 nmol/10⁶ AM (Figure 2). Release of LDH by AM, in contrast, was similar in each group. Alveolar macrophages from NS released 9.2 ± 1.2% of original cell ferritin content over 20 h, whereas AM from LS released 12.9 ± 2.6% and AM from HS released 15.4 ± 2.4% of total cell ferritin stores. Release of LDH by AM over 20 h in culture was 9.6 ± 2.3%, 10.2 ± 2.8%, and 10.4 ± 3.0% for NS, LS, and HS, respectively. Evaluation of the kinetics of ferritin release by AM indicates that there is a more rapid early release followed by a slower late release. In the iron-loaded AM group, for example, 50.3 ± 4.7% of ferritin release occurred during the initial 4 h of culture.

There was a significant correlation between BAL ferritin content and ferritin release *in vitro* over 20 h by AM recovered from smokers (LS and HS, r = 0.72). This correlation was greater for LS (r = 0.91) than for HS (r = 0.52). The overall correlation between *in vitro* release of ferritin and BAL ferritin for all subjects (NS, LS, and HS) was significant (r = 0.80, $p < 0.001$, Figure 3).

Release of transferrin by AM was similar for AM recovered from nonsmokers and both groups of smokers (Table 3).

Concanavalin A-Binding Ferritin in BAL Fluid and AM Culture Supernatants

Normal human serum contains a high proportion of ferritin which binds to concanavalin A. Prior studies suggest that this binding is caused by carbohydrate residues that are added to ferritin during the process of cellular secretion. In contrast, ferritin that is released following tissue injury does not demonstrate binding to concanavalin A. To assess whether ferritin recovered in BAL fluid was derived from cell secretion or cell injury, the proportion of BAL ferritin binding to concanavalin A was determined. In nonsmokers, the ferritin present in BAL fluid did not demonstrate any detectable binding to concanavalin; however, the amounts of ferritin being evaluated in these samples might be near the lower limit of detection of the assay method being used. In contrast, 32 and 52% of ferritin in BAL fluid demonstrated binding to concanavalin A in LS and HS, respectively (Table 2). The small amount of ferritin released *in vitro* by AM recovered from NS demonstrated little binding to concanavalin A (2 ± 1%). A higher proportion of ferritin binding to concanavalin A was present in media conditioned by AM recovered from LS (17 ± 6%) and HS (25 ± 5%). In separate studies we found that ferritin obtained by sonicating AM demonstrated less than 5% binding to concanavalin A for both smokers and nonsmokers.

DISCUSSION

In agreement with previous studies, the current study confirms the presence of an increased lower respiratory tract burden of iron and ferritin in cigarette smokers, including both increased intracellular iron and ferritin in AM, and increased concentrations of extracellular iron and ferritin in alveolar epithelial fluid (2–5). Prior studies indicate that AM are capable of both taking up iron and releasing iron bound to ferritin or to transferrin (6, 12). In order to assess whether AM release of iron and ferritin occurred in these smokers, we quantitated *in vitro* release of iron, ferritin, and transferrin by AM recovered from smokers and nonsmokers. Our findings indicate that AM recovered from smokers released increased amounts of iron and ferritin, but not transferrin, compared with nonsmokers. The increased release of ferritin was greater in heavy smokers than in light smokers, and was most evident in smokers with an AM iron content greater than 10 nmol/10⁶. In these iron-loaded AM, release of ferritin during the initial 4 h of culture was substantially greater than release of LDH, suggesting that release was not solely a result of passive cell leakage. The amount of ferritin released *in vitro* by AM over 20 h also correlated with concentrations of ferritin present in BAL fluid, supporting the concept that AM are a source, at least in part, of increased iron and ferritin concentrations present in alveolar lavage fluid recovered in some cigarette smokers.

Ferritin is the primary iron storage protein and transferrin the primary iron transport protein in human tissues. In the lungs, transferrin is normally present in alveolar fluid in concentrations much higher than ferritin (4). Transferrin binds iron tightly, effectively inhibiting iron-catalyzed oxidant injury (20). Prior studies have suggested that superoxide anion, generated by neutrophils, is capable of mobilizing iron from ferritin, thereby providing catalytic iron for hydroxyl radical formation (21). Other studies suggested that the capacity of superoxide to release iron from ferritin may be limited (22). Recent studies, however, indicate that iron bound to ferritin can be reductively released by reducing agents present

in cigarette smoke, particularly the polyhydroxybenzenes, catechol and hydroquinone (7). In smokers, therefore, extracellular ferritin-bound iron may be capable of contributing to lung oxidant stress if mobilized by reducing agents present in cigarette smoke.

The increase in release of ferritin and iron *in vitro*, but not transferrin, by smokers' AM suggests that much of released iron was bound to ferritin. However, some iron may have been released by AM directly to transferrin present in culture media that was supplemented with calf serum. Studies with animal macrophage populations have demonstrated increased *in vitro* release of ferritin-bound iron following iron loading. Saito and colleagues demonstrated the rat peritoneal macrophages, following phagocytosis of immunosensitized red blood cells, release 20 to 60% of acquired iron back to culture medium over 24 h with approximately 40% of released iron complexed to transferrin and 60% complexed to ferritin (14). The release of substantial amounts of transferrin-bound iron by this macrophage population contrasts with the lack of any increase in release of transferrin-bound iron by the iron-loaded AM recovered from smokers. This difference in release of iron-binding proteins may be related to different capacities of macrophages to synthesize transferrin, because human AM can synthesize ferritin, but not transferrin, whereas AM from mice, for example, have been shown to synthesize both iron-binding proteins (23). Prior studies by Custer and colleagues also noted release of iron stores by human AM that had been iron loaded *in vitro* with a rapid early phase of release followed by a slower phase (12).

The concentrations of extracellular iron and ferritin that we found in alveolar lavage fluid recovered from heavy smokers (49.7 ± 5.0 ng/ml iron, 313 ± 88 ng/ml ferritin) were similar to concentrations previously reported by Thompson and colleagues in alveolar lavage fluid from smokers with chronic bronchitis (48.3 ± 10.5 ng/ml iron, 456.0 ± 94.5 ng/ml ferritin) (4). The concentration of ferritin we found in alveolar fluid recovered from normal subjects, however, was significantly lower (9.9 ± 3.1 ng/ml versus 171.0 ± 94.1 ng/ml). The reason for this difference is unclear; however, the control subjects we evaluated were younger, and age-related variables may influence iron and ferritin concentrations in the lower respiratory tract.

A study by McGowan and Henley evaluated *in vitro* release of ferritin over 24 h by AM recovered from smokers; their findings suggested that ferritin release from smokers' AM was largely due to passive leakage (3). Our findings with regard to ferritin release by AM that contain low amounts of iron, whether from nonsmokers or smokers, are consistent with a passive leak of ferritin. Several lines of evidence, however, suggest that *in vitro* release of ferritin by AM recovered from some smokers is not due solely to cell leakage. First, we found that smokers' AM with increased iron content (> 10 nmol/10^6 AM) release a greater percentage of cell ferritin stores over 4 h than of cell LDH stores (as demonstrated in Figure 2). Second, ferritin release by these AM is more rapid during the initial 4 h of culture than during the subsequent 16 h, when cell death and leakage of ferritin would be more likely. Third, some ferritin released *in vitro* by smokers' AM, as well as ferritin in BAL fluid recovered from smokers, was glycosylated as demonstrated by binding with concanavalin A. These findings do not rule out the possibility that release of ferritin by AM is due, in part, to "leaking" from AM. The correlation between *in vitro* release of ferritin by AM and concentrations of ferritin present in BAL fluid suggests, however, that these findings reflect physiologic processes of iron and ferritin release by AM that occur within alveolar structures of cigarette smokers.

The source of the increased iron burden present in the lower respiratory tract of cigarette smokers remains unclear. Increased lung iron burden may be a result, at least in part, of increased alveolar deposition of iron present in cigarette smoke, which may be as much as 1.12 μg of iron per pack of cigarettes smoked (4, 24). Additional iron may also be derived from increased cell turnover that occurs in the lower respiratory tract of smokers. It has also been shown that tobacco smoke has siderophoric activity and is capable of delocalizing iron and driving iron across cell membranes (25). In addition, prior studies by McGowan and colleagues demonstrated that although there were no differences in iron uptake by AM from smokers and nonsmokers, smokers' AM released newly internalized iron more slowly than AM from nonsmokers (2). Our data, demonstrating greater total release of iron by AM from smokers, together with earlier studies, would suggest that smokers' AM retain newly acquired iron more avidly than nonsmokers' AM, but as total iron content increases, smokers' AM may release iron stored in cytosolic ferritin.

The clinical significance of increased release of ferritin-bound iron by AM in some smokers is uncertain. Ferritin is known to effectively sequester intracellular iron and thereby limit the potential for oxidative cell damage. The capability of extracellular ferritin to effectively sequester iron on the alveolar surface of smokers is less clear. The findings of Olakanmi and colleagues that BAL fluid from smokers did not catalyze hydroxyl radical formation in response to superoxide exposure to any greater extent than BAL fluid from nonsmokers suggests that superoxide is not capable of mobilizing iron from alveolar extracellular ferritin (6). A number of other reducing radicals, however, are capable of releasing ferritin-bound iron including the polyhydroxybenzenes, hydroquinone and catechol, which are present in cigarette smoke. Studies by Moreno and colleagues demonstrated that release of iron from ferritin by aqueous extracts of cigarette smoke was not related to the presence of superoxide, but correlated with concentrations of polyhydroxybenzenes (7). These components of cigarette smoke might release iron from ferritin *in vivo* with potential for hydroxyl radical formation. Ferritin-bound iron in alveolar structures of smokers may also be capable of propagating lipid peroxidation by mechanisms that do not involve hydroxyl radical formation (26). Lipid peroxidation products have been shown to reduce elastase inhibitory capacity of alveolar lining fluid in humans (27). The potential role of increased alveolar extracellular ferritin in stimulating lipid peroxidation in smokers may be enhanced by decreased alveolar concentration of vitamin E in cigarette smokers, an important inhibitor of lipid peroxidation (28).

In summary, our studies of *in vitro* release of iron and iron-binding proteins suggest that the increased concentrations of extracellular alveolar iron and ferritin in alveolar structures of some cigarette smokers are derived, at least in part, from increased release of ferritin and iron by iron-loaded AM. Because prior studies by Olakanmi and colleagues demonstrate that smokers' AM actively take up exogenous iron, our findings indicate that AM in smokers are involved in a dynamic equilibrium with respect to iron metabolism that includes uptake of unbound or transferrin-bound iron and release of cytosolic ferritin-bound iron as AM become iron-loaded. The increased extracellular iron bound to ferritin in alveolar structures of smokers may potentially be reductively released by components of cigarette smoke including hydroquinone and catechol. Further studies are needed to assess to what extent extracellular ferritin-bound iron is mobilized *in vivo* by components of cigarette smoke, or by other reducing agents in cigarette smokers.

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