Detection of Mercury and Other Undetermined Materials in Skin Biopsies of Endemic Pemphigus Foliaceus

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Abstract: A novel variant of endemic pemphigus foliaceus (EPF) was described among individuals in an area surrounding El Bagre, Colombia, South America. The population in this rural mining community is exposed to high environmental levels of mercury, used for gold extraction, as well as other minerals, metalloids, and trace elements (e.g., quartz, rutile, granite, magnetite, and almenite) and ultraviolet radiation. Fifty control subjects and fifty EPF patients in the endemic area were examined for the presence of mercury in skin biopsies and hair, using autometallographic and mass spectroscopic analyses, respectively. Simultaneously, serum levels of IgE were measured, and cutaneous tests for hypersensitivity reactions were performed. Using autometallography, mercuric sulfides/selenides were detected in 14 of 51 skin biopsies distributed similarly in the control and patient groups. However, significantly higher serum IgE levels and mercury concentrations in hair, urine, and nails were found in patients compared with controls. Microscopic analysis revealed mercuric sulfides/selenides concentrated within and around the sweat gland epithelium, as well as in dendritic cells. Five skin biopsies from EPF patients and five from controls that tested positive for the presence of mercuric sulfides/selenides by autometallography were randomly selected for electron microscopic analysis. This analysis revealed a mixed electron-dense and electron-light material closely associated with desmosomes in patients. However, there were intracellular vesicles containing an amalgam of electron-dense and electron-light materials only in the EPF patients. Thus, EPF-affected individuals are exposed to high levels of environmental mercuric sulfides/selenides and other elements. This is the first study reporting mercuric sulfides/selenides in skin biopsies from people living in a focus of EPF, and these compounds may play a role in the pathogenesis of autoimmunity.

Key Words: Endemic pemphigus foliaceus, Cell-adhesion molecules, Trace elements, Musculoskeletal diseases, Desmosomes, Autoimmunity, Bio-elements, Mercury

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A difficulty in the study of autoimmunity is the identification of the possible triggering factor(s) involved in the process.[1] In terms of this issue, endemic pemphigus foliaceus (EPF) is the only described endemic disease observed in a relatively well-defined geographic region.[2,3] This unique feature makes EPF an excellent model system to study the complex interactions between genetics, environment, and the immune system.[2] EPF has been described in foci in the South American rain forest, mainly in Brazil,[2–4] but also in other countries of Latin America (e.g., Colombia)[5–9] and in Tunisia.[10,11] Although in this latter focus no epidemiological studies were performed, it is generally accepted that pemphigus foliaceus (PF) and Brazilian EPF, or fogo selvagem (FS), are characterized by acantholysis and blistering in the upper epidermal layers with deposits of predominantly IgG4 autoantibodies in the intercellular spaces.[12] One major target antigen in these diseases is desmoglein 1 (Dsg1), a desmosomal glycoprotein that belongs to the cadherin family of calcium-dependent cell adhesion molecules, ubiquitously localized in desmosomes.[13,14] The cause of FS in Brazil is unknown; however, environmental risk factors, such as exposure to *Simulium pruinosum,* have been implicated as possible triggering factors for this disease in a host with the proper genetic background.[3,4] Another focus of EPF has also been described in the area around El Bagre, Colombia, South America.[2,5,7] Additional studies from our laboratory resulting from a 10-year epidemiological survey in El Bagre, Colombia, established that this focus in fact represents a new type of EPF with clinical manifestations similar to those

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described for pemphigus erythematosus, better known as Senear-Usher syndrome (a disease resembling a mixture of lupus and pemphigus).[9] El Bagre EPF predominantly affects 40- to 60-year-old males, as well as a few post-menopausal females, and the patients are primarily miners who also engage in agricultural activities.[9] In contrast, Brazilian EPF, or fogo selvagem (FS), primarily affects children and young adults, with the highest incidence at 10 to 30 years of age, and with both sexes equally affected.[2,3] The El Bagre EPF has the following clinical features: (1) hyperpigmented plaques and macules mainly on the face and trunk, predominating in the mid-chest area; (2) an erythematous butterfly-like macular lesion with fine scaling and erosions on the face; (3) pruriginous or hyperkeratotic patches with crusting mainly on the presternal and interscapular seborrheic regions and armpits; (4) occasional scarring and scabbing of the scalp, resembling a false tinea amiantacea; (5) hyperpigmentation of previous lesions (seen mostly in people demonstrating clinical control); (6) the presence of pustules around scaling lesions; and (7) an actinic conjunctivitis-like condition. This last feature appears mainly in cases with high clinical and immunologic activity. Histopathologic changes in lesions of El Bagre EPF mostly resemble those seen in pemphigus foliaceus (Abreu *et al,* manuscript in preparation). Subjects who fulfilled most or all of the above clinical criteria and have immunopathologic findings consistent with pemphigus were diagnosed with El Bagre EPF (Abreu *et al*, manuscript in press).

Metalloids, minerals, and trace elements (e.g., quartz, rutile, granite, magnetite, and almenite) are ubiquitous in El Bagre, and in particular, mercury is used daily for gold extraction processes.[15] Mercury has been demonstrated to trigger autoimmune phenomena in rats and mice that possess the proper genetic background.[16,17] Therefore, we searched for the presence of mercuric sulfides/selenides in the skin and hair of patients from the rural mining community of El Bagre to determine a possible correlation between mercury exposure and/or its presence in skin and the development of EPF. Mercury has also been shown to increase serum IgE levels in genetically predisposed rats and mice, which can then develop autoimmunity.[16] Therefore, serum IgE levels were examined to identify possible connections to the immune hyper-reactivity seen in EPF.

METHODS

Study Subjects

All patients participated freely in this study and signed a consent form. In addition, the Institutional Review Board and Human Assurance Committee approved this study in accordance with the Scientific and Ethics committees of the respective institutions. A careful history was obtained from all patients and controls. During examinations of subjects, clinical diagnosis of any skin diseases or allergic processes, as well as a complete medical history, were obtained by a trained dermatologist and by a trained allergist. Two skin biopsies were taken from each subject; in the case of visible dermatitis and/or pemphigus lesions, the biopsies were taken in part from lesional skin and in part from normal-appearing skin. Most biopsies were taken from the chest area. In the controls with no skin lesions, the biopsies were also taken from the chest area, and then fixed in 10% formalin. These samples were processed by routine H & E staining. The clinical diagnosis was confirmed by histopathological analysis by a trained dermatopathologist.

Since it has been demonstrated that some dermatoses can increase percutaneous absorption of mercury,[18] we included controls with other skin diseases (25 of 50) as well as individuals with no dermatoses. Therefore, in addition to 25 normal individuals, the following disease controls were used: atopic dermatitis (5 of 50), actinic keratosis (7 of 50), lichen planus (1 of 50), vitiligo (5 of 50), cutaneous amyloidosis (1 of 50), basal cell nevus syndrome (1 of 50), nodulocystic acne (1 of 50), and psoriasis (4 of 50). Patients and controls were matched by age, gender, living area, and work activities. Skin biopsies were sent to the Department of Oral Pathology, Malmö University, Malmö, Sweden for autometallographic analysis and to the Department of Dermatology at Wayne State University for electron microscopic analysis. Serum was also collected and stored at −80°C until analysis.

Mercury Detection in Hair, Urine, and Nails

Since mercury can be toxicologically detected in hair, nails, and urine with similar confidence, we measured mercury in the hair of all participants and in the nails and urine of half of them. After extensive washing, 25 mg of hair and/or nails were cut and degreased with acetone. Hair and nail samples were packed separately in ash-free paper and incubated in a solution of nitric and sulfuric acid followed by a solution of potassium permanganate to destroy organic material. Excess potassium permanganate was removed with hydroxylamine chloride.[19] Following this, tin chloride was added to extract the mercury from the hair or nails. Mercury was measured in an atomic absorption spectrophotometer (MAS 50). The World Health Organization (WHO) accepts a permissible level of mercury in hair of 7 parts per million (ppm.)[19]

The total mercury content in urine was determined by mass spectrometry with the so-called cold vapor method after on-line oxidative treatment of the sample in a microwave oven.[20] Use of a $KBr/KBrO_3$ mixture, microwave digestion, and a final oxidation with $KMnO_4$ assures complete recovery of the organic forms of mercury. Quantitative recoveries were obtained for phenyl mercuric chloride, dimethyl mercury, mercuric acetate, and methyl mercuric chloride. The determined normal limit was similar to that described for hair.

Quantification of Total IgE Serum Levels

Since mercury can increase serum IgE levels in genetically predisposed rats and mice, which can then develop autoimmunity,[16] we tested for serum levels of this molecule in the study subjects. Using a commercial ELISA kit (AlaSTAT Total IgE kit, Diagnostic Products Corporation, Los Angeles, CA), normal IgE levels in rural areas of underdeveloped countries is defined as 300 international units per liter (IU/L) or less.[21] Since parasitic diseases are one of the most common causes of IgE elevations in people living in rural areas, all subjects received anti-parasitic medicines 2 and 4 weeks before testing for serum IgE levels. Serum tests were performed after serial stool tests negative for parasites.

Cutaneous Test

Atopy is another common cause of an increase in serum IgE levels. We tested for a cutaneous response to different allergens by injecting *Dermatophagoides (D. pteronyssinus* and *D. farinae)*. Approximately 20 to 30 *u*l of these agents (AbelloALK, Madrid) at a concentration of one biologic unit were applied intradermally (1 UI/ml I.D.) in the lateral area of the arm, using an insulin syringe. Positive (histamine chlorohydrate 1:10,000) and negative (saline solution) controls were also used. A positive result was indicated by erythema with a diameter of greater than 5 mm and/or edema.[22]

Autometallography

To detect mercury in skin, the method of Danscher and Møller-Madsen was used to visualize tissue-bound mercury in paraffin sections.[15,17,18] Briefly 3-μm sections were coated with 0.5% gelatin, air dried, and subsequently developed at 26°C for 1 hour in the dark.[23] The developer contained gum arabic, sodium citrate buffer, hydroquinone, and silver lactate. The gelatin-coated sections were washed in 40°C tap water and rinsed in distilled water, followed by incubation in 5% sodium thiosulfate to suppress background staining. Sections were prepared in quadruplicate, one of which was lightly counterstained with hematoxylin and eosin. The autometallographic technique reveals silver sulfides/selenides, metallic silver, and gold in addition to mercuric sulfides/selenides. To differentiate between mercury and silver, additional sections, mounted on poly-L-lysine-treated glass slides (Polysine, Menzel, Germany), were incubated in a 1% aqueous potassium cyanide (KCN) solution for 2 hours to remove silver prior to autometallographic development.[23–25] To exclude the presence of gold, additional sections on poly-L-lysine-coated slides were instead pretreated with 10% KCN for 15 minutes.[23–25]

Electron Microscopic Analysis

Specimens were fixed in 5% glutaraldehyde and 1% osmic acid in phosphate buffer, pH 7.2, and embedded in araldite. The thin sections were stained with 1% uranyl acetate and lead citrate and examined in a Hitachi H-300 electron microscope as previously described.[26,27]

Statistical Evaluation

Previously codified original data, tabulated in the Epi-Info program for distribution frequencies and associations, were graphed with the Harvard Graphics package.

RESULTS

Serum IgE and Mercury Levels in Hair, Nails, or Urine

In all cases, EPF patients showed significantly higher levels of both IgE and mercury levels than the control group ($P < 0.05$) (Fig. 1). Patients for whom mercury levels were also determined in nails or urine (a 24-hour collection), demonstrated a high correlation with mercury levels measured in hair (Fig. 1). Although a slight increase in mercury levels of the group of controls with dermatoses was observed compared with controls with no dermatosis, this difference was not statistically significant.

Autometallographic Analysis

Our results showed that in 7 biopsies from EPF patients and in 7 controls mercuric sulfides/selenides were visualized as a dark staining. In the 7 positive skin biopsies from controls, 1 belonged to a patient with psoriasis, 1 to a patient with oral lichen planus, and 1 to a patient who had suffered from severe mercury intoxication. The most common localization of mercuric sulfides/selenides was in the secretory epithelium of the sweat glands (Fig. 2 A, B, and C). The skin biopsy from the control patient who had previously suffered from mercury intoxication showed numerous mercuric sulfide/selenide-loaded dendritic–like cells throughout the dermis. A similar positive epidermal staining was observed at the basement membrane in both patients and controls. Most studies using metallographic analysis have been done in Caucasians because it can be difficult to differentiate the melanin pigment from the mercuric selenides/sulfides after staining with silver-based stains. Nevertheless, mercuric selenides/sulfides were observed using the autometallographic technique, in which incubation with 1% aqueous potassium cyanide (KCN) solution for 2 hours to remove silver prior to autometallographic development is performed. Thus, these 2 tests allowed for the simultaneous detection of melanin and mercuric selenides/sulfides.

Mercuric sulfides/selenides were also detected in separate serial sections mixed with melanin grains inside melanophage-like cells in both the epidermis and the dermis. A great number of such cells were detected around the blood vessels and around the sweat gland structures. In addition, mercury was detected inside of sebaceous gland cells.

Cutaneous Test

Only 1 EPF patient tested positive for *D. pteronyssinus* and *D. farinae*. In addition, no statistically significant differ-

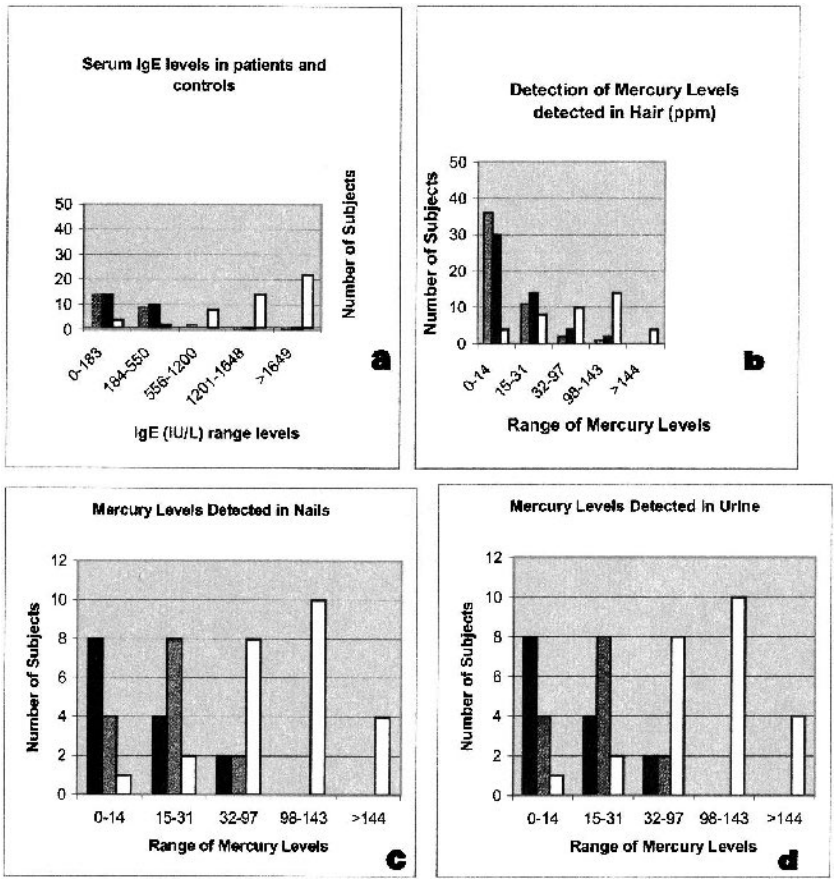

FIGURE 1. A, Quantification of total serum IgE levels and mercury. Serum IgE levels were determined in 25 of 50 normal individuals with the dermatoses described in methods (gray bars), in 25 of 50 control individuals without dermatoses (black bars), and in 50 EPF patients (white bars). The results are shown in terms of the ranges of IgE international units per liter (IU/L) from 0–183, 184–550, 556–1200, 1201–1648 and greater than 1649. The EPF patients showed increased levels of IgE compared with controls ($P < 0.05$). B, In the same number of subjects, we detected mercury levels in hair (ppm). According to the World Health Organization (WHO) a permissible level of mercury in hair is 7 ppm. The EPF patients showed increased levels of mercury compared with controls ($P < 0.05$). In panels C and D, we randomly tested 28 of 50 EPF patients, 14 of 25 control individuals with, and 14 of 25 individuals without dermatoses to determine mercury levels in nails (panel C) and urine (panel D). EPF patients exhibited significantly elevated mercury levels. A slight increase of mercury levels in the group of controls with any dermatoses was observed compared with controls with no dermatoses, as described in the text, but this was not statistically significant.

ences were observed in terms of personal or familial history of allergy or atopy (respiratory, skin, or gastrointestinal reactions) between EPF patients and controls.

Electron Microscopic Analysis

Five EPF and five control skin biopsies that showed mercury in the skin by autometallography were examined by electron microscopy. We observed a mixture of electron-dense and electron-light materials amalgamated in the intercellular spaces of the keratinocytes, mainly at the granular layer, in the 5 EPF patients. These deposits were in contact with the intercellular junctions, where one of the autoantigens in pemphigus foliaceus (desmoglein-1) is located (Fig. 3 A through F). We observed 2 predominant types of vesicles: one inside of the cells and another abutting to the intercellular space. These vesicles contained 2 or 3 unconventional electron-dense and electron-light materials and were clearly different from other physiologically known dense bodies. Serial sections of different magnifications are shown in Figure 2 (Fig. 2 A through F). In the skin biopsies from controls that also tested positive for mercury by autometallography, no alterations in the epidermis were detected by electron microscopic analysis.

DISCUSSION

Endemic clustering of autoimmune diseases following xenobiotic exposures reinforces the hypothesis that these diseases might be secondary to genetic, hormonal, and environmental factors.[1] While the existing correlation between autoimmunity and environmental agents has not yet been well established, some possible predisposing environmental agents have been implicated, including mercury, iodine, vinyl chloride, canavanine, organic solvents, silica, L-tryptophan, particulates, trace elements, ultraviolet radiation, and ozone.[1] Mercury is commonly used to amalgamate gold, thereby facilitating its extraction during mining activities. Mercury also amalgamates multiple trace elements and can compete with calcium in calcium-dependent proteins in an allosteric manner.[28]

Desmogleins are also calcium dependent and may be affected by mercury. This metal, as well as other minerals, metalloids, and trace elements, is also present in many products, such as fertilizers, pesticides, and herbicides, employed in farming activities in this endemic area of pemphigus.[15] The El Bagre area also has special environmental conditions, such as its acidic soil, large amounts of waste ash products from forest

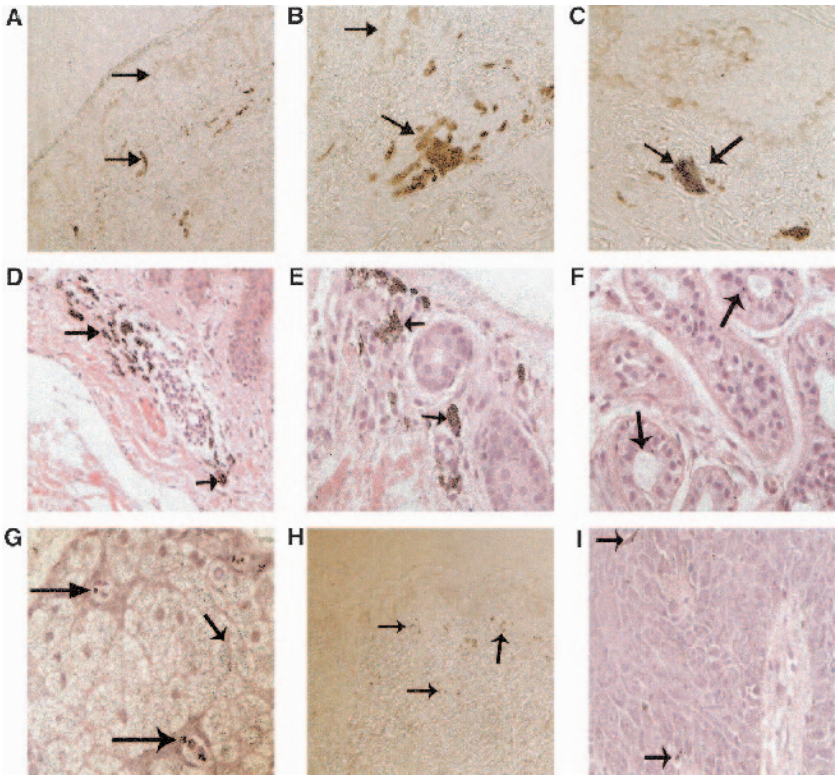

FIGURE 2. A through I, Autometallographic staining of several skin sections taken from EPF patients. In most specimens, the lower epidermis and the dermis close to the basement membrane zone (BMZ) contained abundant melanin, most likely because the majority of people in this area are mestizo (Indian/white), Zambo (Indian/black), Indian, or mulatto (white/black). A, The upper arrow indicates melanocytes and melanin-laden keratinocytes at the BMZ and the lower arrow points to a group of melanophage-like cells located near the superficial dermal vessels at a magnification of 10×. B, A similar area at higher (200×) magnification. C, Indicated by the two arrows are melanophage-like cells filled with mercuric selenides/sulfides (and perhaps other metalloids and trace elements) that can be detected by the autometallographic technique (lower arrow) and melanin-like pigment distributed in the epidermis and in the superficial dermis (upper arrow) (200×). No other such melanophage-like cells were found in any of the other subjects of the study. In terms of melanin, visualization requires staining with a silver-based stain such as Fontana-Masson or DOPA (which detects tyrosine hydroxylase activity). In fact, melanocytes appear clear in sections without such staining. Using Fontana-Masson and silver staining, we detected melanin also. Nevertheless, the technique used for autometallography removes silver since the incubation with KCN removes such staining. Positive epidermal and basement membrane staining for melanin was observed in both patients and control individuals. However, dihydroxyphenylalanine (DOPA staining) was negative in these cells in the dermis (data not shown). No cluster of differentiation markers were examined in the dendritic cells in the dermis by immunohistochemistry. Thus, the identification of these cells is not possible. D, Specimens in which mercuric selenides/sulfides (arrows) were visualized using autometallography were counter-stained with H & E and photographed at 200× magnification. E, Using autometallographic analysis counter-stained with H & E, a group of melanophage- like cells around the sweat glands are shown filled with melanin and mercuric selenides/sulfides (arrows) (400×). F, This picture is similar to panel E, but photographed at higher magnification (400×). Fine deposits of mercury are observed inside the sweat gland epithelium (arrows). Panel (G) shows two types of deposits disclosed by autometallographic staining: a finer grained material spread throughout the sebaceous gland (small arrow) and a larger grained and more localized material (large arrow) within individual cells (400×). H, The entire superficial dermis close to the basement membrane area contained abundant dendritic cells-like cells filled with melanin and/or mercuric/selenides (arrows) (100×). Panel (I) shows similar dendritic-like cells between keratinocytes (arrows) (630×).

fires, wide jungle deforestation, extremely high temperatures and humidity, thunderstorms, and microbe-mineral-metalloid interactions.[15] These factors may increase environmental levels of mercury and other heavy metals, metalloids, minerals, and trace elements.[23] Indeed, previously we reported that 64.2% of the patients affected by EPF were exposed to mercury, and perhaps other trace elements, directly by mining or indirectly by fumigation products that contain mercury.[5,6] In addition, mercury pollution has been reported in the biotic chain, including humans, in El Bagre and neighboring municipalities.[15] The Brazilian FS focus shares several environmental features with the El Bagre EPF focus, such as severe deforestation and extensive mining of rich geological resources.[15,29–31] Our study revealed increased levels of mercury in hair as well as the presence of mercuric selenides/sulfides in sweat glands. Thus, we hypothesize that mercuric selenides/sulfides, minerals, metalloids, and other trace elements could be involved directly or indirectly in triggering EPF disease in a genetically predisposed individual. Alternatively, mercury exposure may increase the incidence of a late mutation of some molecules after exposure to chronic ultraviolet radiation, as occurs in some strains of mice.[32] Patients with EPF have disruption of the skin barrier, and increased mercury uptake has been observed in the skin of patients with various types of dermatitis, such as active atopy, psoriasis, and lichen planus.[18]

Regarding the localization of mercuric sulfides/selenides in skin, it was previously reported that in normal and eczematous skin, 10 to 15% of this metal could be absorbed after topical application. After skin exposure, mercury can be

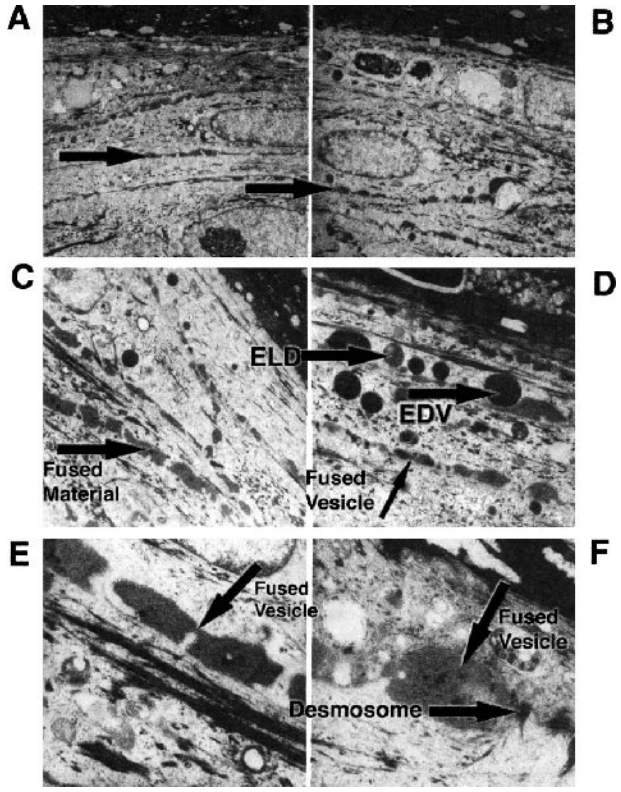

FIGURE 3. A through **F**, Electron microscopic analysis of skin biopsies from EPF patients from El Bagre. Panels (**A**) and (**B**) show the presence of an aligned material located at the intercellular spaces of the keratinocytes (arrows) (plate 4000×, print 12000× f 5). Panel (**C**) illustrates a higher magnification of the same material (plate 5000×, print 15000× f 5). **D**, Under still higher magnification, the fused material was localized to intercellular junctions and the vesicles. The arrows indicate the electron-light (ELV) and electron-dense vesicles (EDV) as well as the fused material at the intercellular junctions of the keratinocytes (plate 15000×, print 45000× f 5). **E**, The material contained in these vesicle inclusions seems to be extruding into the intercellular spaces (plate 15000×, print 45000× f 5). **F**, A higher magnification (plate 15000×, print 45000× f5) of the fused materials indicates the possibility that this may be an amalgam of electron-light and electron-dense materials in close contact with a desmosome. All tissue samples were fixed and processed as described in the Methods section.

detected extracellularly, usually closely associated with desmosomes and external surfaces of cell boundaries.[33,34] We detected a mixed material with an electron density similar to that of mercury in close proximity to the desmosomes, where one of the target antigens of pemphigus disease is located.[13,14] As a comparison, we tested skin samples from the group of controls with dermatoses, which also tested positive for mercury by autometallography but found no materials in the desmosomes similar to those observed in the EPF patients. By studying FS skin and mucosal biopsies, Sotto et al[35] have also reported the presence of "corpuscles" that were hypothesized to be lamellar bodies or transverse cuts of cell interdigitation, but their nature remains unsolved. Larger EM studies may provide insight into these particles, in both the Brazilian and this new variant of EPF. In addition, future studies will be necessary to identify the electron-dense and electron-light materials using atomic mass spectroscopy or x-ray elemental analysis and transmission electron microscopy. Nevertheless, we speculate that the binding of mercuric sulfides/selenides and/or other electron-dense and electron-light materials either activates metalloenzymes or alters sulfur groups, to produce a chronic molecular alteration that results in an autoimmune response.[36] Thus, we hypothesize that these metals, metalloids, and/or other trace elements can amalgamate and bind cellular molecules in vivo, thus producing changes in their molecular structure that are recognized by the immune system as foreign, thereby activating the alternative complement cascade and other inflammatory molecules.

Mercury can also be detected in intact skin by electron microscopy using a gold chloride stain.[33,34] By this method after topical application, mercury was detected in skin nuclei, tonofilaments, mitochondria, melanosomes, desmosomes, intercellular spaces, and cell membranes as well as bound to many proteins, like metallothionein and lipoproteins.[33,34] Other studies testing for the behavior of externally applied radioactive mercury in normal and psoriatic skin and seborrheic dermatosis demonstrated that diseased epidermis retains this metal more effectively than normal skin.[18] Thus, the greater amount of mercuric sulfides/selenides present in skin of EPF patients, as well as controls with dermatoses might be due to a reduced barrier function that allows more mercuric sulfides/selenides to be absorbed compared with controls without skin conditions. Interestingly, in the El Bagre population, we observed an increased rate of lichen planus compared with other rural areas of Colombia, suggesting that an environmental factor such as mercury might also be increasing this disease simultaneously with the increased incidence of EPF (Abreu *et al*, manuscript in press).

Although the increased serum IgE levels observed may indicate a response to an infectious agent(s) in EPF patients, we believe that the high levels are unlikely to be the result of type I allergy (an IgE-antibody-mediated hypersensitivity disease), or other common causes, since there was no difference in the incidence of IgE myeloma, smoking, active parasitic infections, or atopy (personal or family-related) between EPF and controls. Indeed, an increase of serum IgE levels has also been described in animal models after exposure of susceptible mouse strains to mercury .[16,17] In addition, it was previously shown that some pemphigus sera also exhibited increased IgE levels compared with controls, for instance, sera of patients with FS compared with PV patients or healthy individuals.[37,38] It was also demonstrated that in FS the desmoglein-1 specific T-lymphocytes are of the T_H2 type with cytokine profiles that

promote IgE secretion.[39] Another study also showed increased serum levels of IgE in patients with FS, but an additional skin test using air fungi was performed and resulted in a slightly elevated cutaneous response in the FS group compared with the controls.[37] In our study, we did not test for the presence of fungi or fungi-produced toxins, which can also increase IgE sera levels, as in the case of pulmonary aspergillosis or *Fusarium*.[40]

On the other hand, in combination with the observation of autoimmune IgG4 antibodies in EPF serum, another possible explanation of the high serum IgE levels in patients with EPF is isotype switching between IgG4 and IgE. In experimental models, (i.e., Brown Norway rats) mercury may induce an autoimmune syndrome in genetically sensitive strains.[41] The syndrome is characterized by the deposit of linear IgG along the glomerular basement membrane, antinuclear antibodies, high serum levels of IgE, IgG1 and IgG4, and dermatitis.[41] The mechanisms by which mercury generates this syndrome are as yet unknown, but similar changes also appear in animals treated with D-penicillamine or gold salts.[42] Moreover, PF can also be triggered by D-penicillamine.[43] Thus, a combination of several factors may be necessary to break self tolerance and result in xenobiotic-induced autoimmunity. Such factors likely compromise endogenous components such as T-cell receptors, regulatory T-cells, and human leukocyte antigen, but currently these ideas are speculative.

Endemic and epidemic diseases occurring in an acute or chronic manner, in a restricted, relatively well-defined geographic area, and arising as a result of autoimmune, degenerative and/or metabolic disorders, is not uncommon among people who may be genetically predisposed to react to different environmental factors. Examples of such diseases include pink disease (acrodynia), the increased autoimmunity associated with the Minamata Bay disaster and methyl mercury poisoning, generalized disorders of ectodermal tissue following prenatal exposure to polychlorinated biphenyls reported in Taiwan and Japan, the Apallic syndrome, Uric (Kashin-Beck) disease, eosinophilia-myalgia syndrome, hip disease seen in the Mseleni of Northern Zululand, Handigodu disease in Karnataka, India, Urov disease (osteo-arthrosis endemic in Siberia and China), Keshan disease, and the hard metal lung diseases such as silicosis.[44–55] In all these cases, a multidisciplinary approach for understanding the syndromes is necessary, since a multifactorial genetic and environmental cause appears probable.

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