Granulomatous Interstitial Pneumonia in a Miniature Swine Associated with Repeated Intravenous Injections of Tc-99m Human Serum Albumin: Concise Communication*

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Albumin lung-scanning agents have a proven high degree of safety, with the only contraindication to their use being allergic hypersensitivity. We have used these agents to investigate the physiologic effects of high G_z acceleratory forces on pulmonary perfusion using the miniature swine. Multiple doses of human macroaggregated albumin and human-albumin microspheres were given to a miniature swine at various levels of centrifugal acceleration over a 6-wk period. The dosages given were the same per kilogram as those used for routine clinical human studies. The animal subsequently died from a severe granulomatous interstitial pneumonia. The granulomatous lesions suggest that the pathogenesis may have involved a cell-mediated delayed hypersensitivity. This interstitial pneumonia may represent the end point in a chronic hypersensitivity response to the human-albumin lung-scanning agents.

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Adverse reactions to lung-scanning agents are not common, with the only contraindication to their use being a previous allergic response to them (*1*). The acute hypersensitivity reactions have been attributed to the albumin carrier during use of human macroaggregated albumin or human-albumin microspheres, and to the ferric iron, rather than ferrous iron, if hydrated iron hydroxide is used (*2,3*). Pathologic changes have also been observed with carriers consisting of iron hydroxide macroaggregates (*2,3*) and human albumin (*4,5*). These previous investigations have focused on acute toxicity to high-particulate dosages or on pathologic changes due to large single doses. The proportion of lung vessels blocked (*6*), and acute toxicity as a function of size and number (*7*), are known. Pulmonary diffusion capacity (*8*) and pulmonary artery pressure (*8,9*) have both been evaluated as indices of acute toxicity to particulate lung-scanning agents.

Histopathologic examinations of lung tissue following injection of human-albumin lung-scanning agents have been sparse. Infarction and hemorrhage shortly after injection of massive doses (*2*), followed by conversion later to minute scars or granulomatous inflammation (*5*), have been observed. Specifically, the endothelial and perivascular lymphoreticular cell proliferation, with associated giant cells, was considered suggestive of early "granulomatosis" inflammation (*5*). Antigenic studies in several animal species revealed sensitization to be low to repeated doses of human-albumin microspheres, although sensitization was possible particularly with soluble human serum albumin (*10*). Overall, these antigenic studies were thought to indicate a low probability of serious patient reactions to repeated injections of human-albumin microspheres. No antigenic responses were noted in patients after as many as eight adminis-

* The animals involved in this study were procured, maintained, and used in accordance with the Animal Welfare Act of 1970 and the "Guide for the Care and Use of Laboratory Animals" prepared by the Institute of Laboratory Animal Resources-National Research Council.

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trations during clinical trials over a 2-yr period (*5*).

In spite of the low probability, serious reactions related to radiolabeled albumin scanning agents remain a distinct theoretical possibility. This report presents a case of a miniature swine that received six injections of Tc-99m macroaggregated human albumin followed by four doses of Tc-99m human-albumin microspheres. The animal died from a severe granulomatous interstitial pneumonia. The possibility that this pneumonia was related to the repeated injections of human-albumin scanning agents is discussed.

EXPERIMENTAL METHODS

Miniature swine have been used as human models for the investigation of high $+G_z$ acceleratory forces (head-to-foot forces) similar to that experienced by pilots flying high-performance fighter aircraft during combat. Experiments were performed on the centrifuge of the USAF School of Aerospace Medicine (USAFSAM). The miniature swine were from a Pitman-Moore strain.† All animals were maintained in individual runs inside a vivarium. Before surgical catheter placement and entry into the research study, routine chest radiographs were taken and found to be normal. In addition, the initial baseline resting pulmonary perfusion scans after surgical placement of the indwelling catheter were normal, so the animal was considered normal upon entry into the study. The radiolabeled albumin scanning agents were the same as those used for routine clinical human lung scanning. Dosages were the same per kilogram as those used for human studies. Injections of human macroaggregated albumin (MAA) and human-albumin microspheres (HAM) were made during centrifugation to levels from $-4G_z$ to $+8G_z$ by a remote-controlled injection system via a chronically implanted Silastic catheter placed in the cranial vena cava, as previously described (*11*). They were given once or twice a week over a 6-wk period. The first six injections were ~2.5 mCi of Tc-99m MAA per injection, and the final four contained ~2.5 mCi of Tc-99m HAM.

Twenty days following the final injection, the animal was found dead in its cage. A complete necropsy was carried out.

PATHOLOGY

Gross. The 34-kg, black, male miniature swine had been dead for ~8-12 hr and was moderately autolytic. The only gross changes not attributable to autolysis were seen in the lungs, which did not collapse, were firm, and had a uniform granular texture. Numerous minute (<1 mm), white, firm, spherical nodules were visible on both the serosal and cut surfaces and were uniformly distributed throughout all lobes (Fig. 1). There were no pleural adhesions, and except for hypostatic congestion on one side, the color was normal.

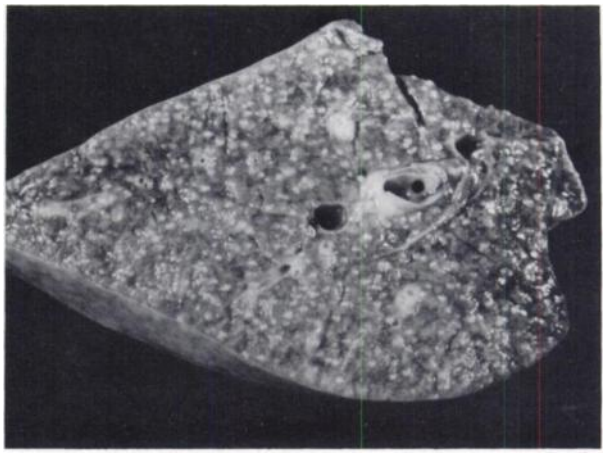

FIG. 1. Numerous white, 1- to 2-mm granulomas are uniformly distributed throughout this section of left diaphragmatic lobe.

Microscopic. Numerous granulomas were randomly and evenly distributed throughout the sections. These lesions appeared to have arisen in alveolar septa and had no association with airways or the larger vasculature. Characteristically, a granuloma had a small central core of necrotic debris and neutrophils surrounded by a zone of macrophages, lymphocytes, and, finally, a thin capsule of mature fibrous connective tissue (Figs. 2 and 3). In many lesions the necrotic core was heavily mineralized, and Langhans' giant cells were frequent in others.

Generally there was no visible inciting agent in the granulomas, but in a few there were round, homogeneous, acellular, eosinophilic, 25- to 30-μm structures surrounded by neutrophils in the center of the granuloma (Fig. 3). Although there was no specific stain for albumin, the round central structures in several of the granulomas stained eosinophilic with H & E, which is

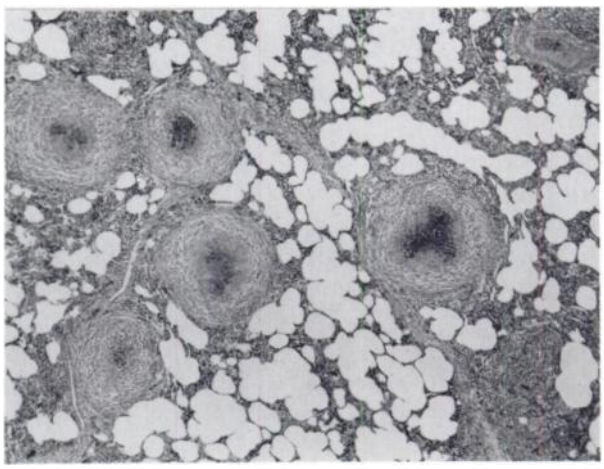

FIG. 2. Representative microscopic section of lung containing several interstitial granulomas. Typically they consist of necrotic or neutrophilic core surrounded by zones of macrophages, lymphocytes, and fibrous connective tissue. Hematoxylin and eosin, X24.8.

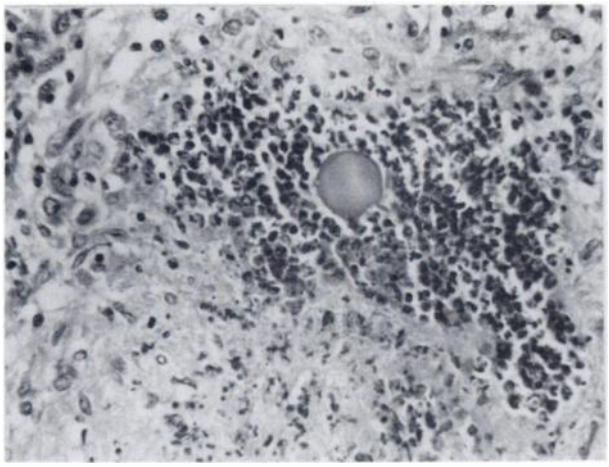

FIG. 3. Center of granuloma contains 28-μm round, eosinophilic structure in focus of neutrophils. Structure is compatible with that of albumin microsphere. Hematoxylin and eosin, X307.

how albumin should stain if it were present. Rarely, granulomas were seen containing small amounts of a cellular, honeycombed, slightly refractile material suggestive of plant origin. Lesions with this type material were primarily made up of giant cells within the fibrous capsule. Special histologic stains for mycobacteria, fungi, and bacteria were all negative. No granulomas or other inflammatory lesions were present in the mediastinal lymph nodes or at any other extrapulmonary site. All other tissues were unremarkable.

DISCUSSION

Agents capable of inducing granulomatous disease usually fall into the categories of infectious agents (fungi and certain bacteria), foreign bodies, parasites, or particulate antigens capable of inducing delayed hypersensitivity reactions, as in tuberculosis. The extremely uniform distribution of lesions in this case, and their interstitial location, are indicative of hematogenous dissemination of the inciting agent. Furthermore, uniformity of size, stage of development, and distribution of lesions suggest that the causative agent was a particulate material of fairly uniform size, which was trapped throughout the pulmonary capillary bed following introduction into the major vasculature. Immunologically a chronic hypersensitivity reaction would appear at the time of the reaction in this animal. After sensitization with the initial doses of human albumin, the high antigenic load of subsequent doses may have induced a very rapid response, possibly resulting in trapping of the particulate within the reaction center. This may have resulted in persistence of the particles within some of the granulomas.

Fungi and bacteria are usually easy to demonstrate in lesions, are not so evenly distributed, and produce a more pyogranulomatous response, rather than distinct granulomata. Parasites are likewise easy to demonstrate, are generally accompanied by eosinophils, and are not known to be so uniformly distributed.

Tuberculosis was ruled out on the basis of uniformity of distribution, failure to demonstrate a primary site, lack of involvement of regional lymph nodes or other tissues, and failure to demonstrate acid-fast organisms in any of the lesions. The resemblance of these lesions to the classic "tubercle" is significant, however, and suggests that the pathogenesis may involve a cell-mediated delayed hypersensitivity reaction such as occurs in tuberculosis.

Factors supporting the contention that the radioactive albumin of human origin is responsible for the lesions seen are: (a) demonstration of structures morphologically compatible with the microspheres in some of the granulomas; (b) the size and method of administration of the microspheres, via i.v. cannulation, would account for the uniform distribution of the lesions; (c) the particulate nature of the protein antigen would be expected to induce a cell-mediated immunologic response (if any immunologic response were illicited) rather than, or in addition to, a humoral antibody response; and (d) the chronic, repetitive administration schedule of the antigen would have increased the likelihood that an immunologic response would occur. Body scans for radioactivity indicate that nearly all of the microspheres are trapped in the pulmonary circulation, which would account for the absence of lesions in other organs, including the regional lymph nodes. Virtually complete trapping of polystyrene microspheres 9 $\pm$ 0.8 μm in the swine pulmonary circulation has been found previously (*11*).

The possibility of a nonspecific foreign-body type of reaction to the microspheres, or to some particulate contaminant, cannot be completely ruled out, but a similar injection schedule of MAA and HAM in other swine produced no such lesions. Undoubtedly some contamination did occur, possibly during routine flushing of the catheter, as evidenced by the very few granulomata containing cellular particulate matter, probably of plant origin. If the entire process were due to this type of gross contamination, however, most lesions would have a larger component of giant cells, and the offending material would be expected to have persisted in more lesions. Moreover, uniformity of particle size would be unlikely and a less uniform distribution expected. Conversely, the absence of the proteinaceous microspheres intact in the lesion core is expected, and the persistence of even a few after several weeks is not fully understood.

The use of *human* albumin in the miniature *swine* would seem, theoretically, to carry additional risk of hypersensitization, although previous work with serial injections of human-albumin microspheres in dogs failed to initiate an antigenic response. Although it was reported to be possible to sensitize guinea pigs with soluble human serum albumin, it was not possible to sensitize

them with human-albumin microspheres. The animal reported in this study received several doses of human macroaggregated albumin and human-albumin microspheres and could well have manifested a unique chronic hypersensitivity to the human serum albumin. We have not seen similar fatal reactions in other miniature swine undergoing the same dosages over similar time periods.

The miniature swine is finding wide application in several areas of biomedical research; therefore, more detailed observations on the response of this animal to various pharmacologic and diagnostic agents are important. The sensitizing of a particular animal to human serum albumin lung-scanning agents with multiple doses may be possible, although not common. These diagnostic agents continue to have an excellent record of safety, with the only clinically adverse observation being related to hypersensitivity. Granulomatous inflammation reactions have been reported to follow large doses of these agents in animals. The pathologic changes reported here, which resemble granulomatous interstitial pneumonia, may represent the end point in a hypersensitivity response.

FOOTNOTE

† Vita Vet Laboratories, Inc., Marion, IN.

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