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Experimental Extrinsic Allergic Alveolitis and Pulmonary Angiitis Induced by Intratracheal or Intravenous Challenge with *Corynebacterium parvum* in Sensitized Rats

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Extrinsic allergic alveolitis and pulmonary sarcoidosis are granulomatous diseases of the lung for which clinical presentation and anatomic site of granuloma formation differ. Extrinsic allergic alveolitis is caused by inhaled antigens, whereas the nature and source of the inciting antigen in sarcoidosis is unknown. To test the hypothesis that the route via which antigen is introduced to the lung contributes to the clinicopathological presentation of pulmonary granulomatous disease, rats immunized with intravenous (i.v.) **Corynebacterium parvum** ***were challenged after 2 weeks with either intratracheal (i.t.) or i.v.*** **C. parvum.** ***The granulomatous inflammation elicited by i.t. challenge predominantly involved alveolar spaces and histologically simulated extrinsic allergic alveolitis. In contrast, the inflammation induced by i.v. challenge was characterized by granulomatous angiitis and interstitial inflammation simulating sarcoidosis. Elevations of leukocyte counts and TNF levels in bronchoalveolar fluid, which reflect inflammation in the intra-alveolar compartment, were much more pronounced after i.t. than after i.v. challenge. Tumor necrosis factor, interleukin-6, CC chemokine, CXC chemokine, and adhesion molecule mRNA and protein expression occurred in each model. In conclusion, i.t. or i.v. challenge with*** **C. parvum** ***in sensitized rats caused pulmonary granulomatous inflammation that was histologically similar to human extrinsic allergic alveolitis and sarcoidosis, respectively. Although the soluble and cellular mediators of granulomatous inflammation were qualitatively similar in both disease models, the differing anatomic source of the same antigenic challenge was responsible for differing clinicopathological presentations. (Am J Pathol 1996, 149:1303–1312)***

Extrinsic allergic alveolitis (EAA, also known as hypersensitivity pneumonitis, farmer's lung, pigeon breeder's lung, air condition lung disease, etc) is a group of pulmonary diseases caused by a wide variety of organic antigens derived from various microorganisms including bacteria, fungi, protozoa, and various plant and animal proteins.[1] EAA is thought to represent an immune reaction following repeated respiratory exposure to an organic antigen.[1] The histopathology of EAA is well defined and is characterized by intra-alveolar and interstitial granulomas as well as bronchiolocentric inflammation.[1,2] Murine models of EAA have been induced by repeated intranasal instillation of *Micropolyspora faeni* (known as the etiological agent for farmer's lung) or by adoptive transfer of *M. faeni*-sensitized spleen cells into naive mice and subsequent intratracheal challenge with *M. faeni*.[3–7]

Sarcoidosis is a systemic granulomatous disease of unknown etiology that involves the lungs in almost all afflicted individuals.[8] Blood vessel wall and intimal granulomas are quite typical for sarcoidosis in contrast to EAA in which granulomas seldom affect the blood vessel.[9] The high frequency of granuloma-

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tous angiitis in sarcoidosis has been documented as ranging from 53 to 69%.[9–11] Venous granulomas are seen in 92% of pulmonary biopsies in which granulomatous angiitis is seen, whereas arterial granulomas are seen in 39%.[9]

The purpose of the present study is to 1) report the development of a model of intra-alveolar and interstitial granulomas with bronchocentric chronic inflammation induced in rats by a single immunizing intravenous (i.v.) injection of *Corynebacterium parvum* followed by a single intratracheal (i.t.) challenge with *C. parvum*, 2) report the development of a model of pulmonary granulomatous vasculitis and chronic interstitial inflammation induced in rats by a single immunizing i.v. injection of *C. parvum* followed by a single i.v. challenge with *C. parvum*, 3) characterize the models by studying the kinetics of inflammatory cell accumulation and expression of cytokines, chemokines, and adhesion molecules, 4) describe the histological similarity of the above mentioned models to the human diseases of EAA and pulmonary sarcoidosis, and 5) discuss the importance of the route via which an antigenic challenge reaches the lung to the resulting clinicopathological features of the pulmonary manifestations.

Materials and Methods

Corynebacterium parvum-*induced Granulomatous Inflammation*

Killed *C. parvum* (RIBI Immunochem Research, Hamilton, MT) was suspended in 0.5 ml of sterile saline solution (3 mg/ml) and was injected via the dorsal vein of the penis at the beginning of experiments as the sensitization. Two weeks after the sensitizing i.v. *C. parvum* injection, rats were challenged with an i.t or i.v. injection of 1.5 mg of *C. parvum*. The i.t. challenge was delivered via intraoral cannulation with a flexible sterile 18-gauge plastic catheter under direct visualization with an endoscopic guide light (Karl Storz, San Clemente, CA). Rats were sacrificed at time 0 (2 weeks after i.v. sensitization) and at 1, 2, 3, 5, 7, and 14 days after the i.t. or i.v. *C. parvum* challenge (n = 6 at each time point). Rats injected i.v. with sterile saline and i.t. or i.v. with saline for challenge were used as controls. At the time of sacrifice, the clamped left lung was removed and frozen for RNA extraction, and the right lung was used for bronchoalveolar lavage (BAL) and histology.

Leukocyte Quantitation in BAL Fluid

BAL was performed as previously described.[12] Absolute numbers of total leukocytes in BAL fluid was enumerated with an automated cell counter (Sysmax, Irvine, CA). Differential counts of leukocyte subsets were determined by counting 200 nucleated blood cells after modified Wright's-Giemsa staining of cytospin slides. The absolute numbers of each leukocyte subset were calculated by multiplying the total and differential and were expressed as the mean ± 1 SD. Probability value was determined by the *t*-test (Systat, Evanston, IL)

Histology and Immunohistochemistry

A midsagittal section of the right lung was taken encompassing the largest surface area. Formalin-fixed, paraffin embedded sections of the lung were stained with hematoxylin and eosin staining and were examined histologically to determine the distribution as well as the severity of granulomatous inflammation. Formalin-fixed, paraffin-embedded lung tissue was used for immunohistochemical localization of ICAM-1 and CD44 expression. After deparaffinization and hydration, tissue sections were treated with 10 mmol/L citrate buffer in a microwave oven (70% power for 3 minutes) for antigen retrieval. CD44 (Pharmingen, San Diego, CA; 1:100 dilution) and ICAM-1 (Caltag, San Francisco, CA; 1:100 dilution) were used as primary antibodies. Standard avidin-biotin-complex (ABC) immunohistochemical staining was performed with biotinylated secondary antibody and 3′,3-diaminobenzidine tetrachloride (Sigma Chemical Co., St. Louis, MO) as the chromogen.

Cytokine Bioassays

Bioassays for tumor necrosis factor (TNF; WEHI 164, subclone 13 bioassay) and interleukin (IL)-6 (B9 bioassay) proteins in cell-free supernatants of BAL fluid were performed as previously described.[13,14] Recovered volume from 7 ml of BAL fluid of the right lung was approximately 5 ml and was relatively uniform in each rat. BAL specimens were serially diluted (six dilutions) in duplicate for a total of 12 wells per specimen. The concentrations of TNF and IL-6 were calculated by averaging all dilutions that fell on the standard curve.

RNAse Protection Assay

Primers for the following cytokines, chemokines, and cell adhesion molecules were prepared based on

these cDNA sequences[15–19]: MIP-1α 5′-CTTGC TGTNCTNCTCTGNACCATG-3′, 3′-CCACAGTAN-AAGGANTGNTTCNCT-5′; MCP-1 5′- GTTAATGC-CCCACTCACCTGCTGC-3′, 3′-ACCTGGTCTTGG-TTCACTCTAGTC-5′; KC 5′- GCGCCCGTCGC-CAATGAGCTGCGC-3′, 3′-GAACGGAACTGGGAC-TTCGGGGGT-5′; MIP-2 5′-CCTGCTGGCCACCAA-CCATCAGGG-3′, 3′-GACTTGTTTCCGTTCCGATTG ACT-5′; IP-10 5′-GTNCGCTG(TC)ANCTGCATC-3′, 3′-TCTACAGACTTAGG(CGT)CTT- 5′; E-selectin 5′-CCCAGTGTGAAGCCTTATCTGCAC-3′, 3′-ACAAG-TTCGGAACTGG AAGGTCCT-5′ GenBank accession number L25527; P-selectin 5′-GGGCAGT-CACTGAATGGC TCTGCC-3′ GenBank accession number L23088; GAPDH 5′-GCCCCCCTGGC-CAAGGTC-3′, 3′-CTGACACCTACCGGGGAG-5′. Oligonucleotides were synthesized on an Applied Biosystems (Foster City, CA) automated DNA synthesizer. Reverse transcriptase polymerase chain reaction or polymerase chain reaction was performed for a total of 40 cycles using lipopolysaccharide-stimulated rat peritoneal macrophages total RNA or a rat lung cDNA library (Clontech Lab, Palo Alto, CA) as the template under the following conditions: denaturing at 94°C, annealing at 54°C, and extension at 72°C. The polymerase chain reaction product was purified on agarose gel and ligated into a pGemT vector (Promega, Milwaukee, WI) and sequenced on an automated DNA sequenator (Applied Biosystems 373A) with a *Taq* DyeDioxy terminator cycle sequencing kit (Applied Biosystems)

Anti-sense riboprobes were prepared by *in vitro* transcription with either T7 or SP6 RNA polymerases (Ambion, Austin, TX) and [^{32}P]UTP. Lung total RNA (5 μg) prepared by acid phenol/chloroform extraction was hybridized with 1×10^5 counts of each riboprobe for 14 to 16 hours at 56°C. Unhybridized RNA was digested with RNAse A and T1 (Ambion) at 30°C for 1 hour, followed by proteinase K digestion (37°C for 30 minutes). After phenol/chloroform extraction and sodium acetate/ethanol precipitation, the mRNA-riboprobe hybrid was denatured and electrophoresed on 6% agarose. The dried gel was exposed to x-ray film (Kodak, Rochester, NY) overnight at −70°C.

Results

Histology

The i.t. injection with *C. parvum* at 2 weeks after i.v. sensitization caused severe neutrophilic inflammation at day 1 (Figure 1), which rapidly subsided and was replaced with predominantly lymphocytic and granulomatous inflammation by day 2. The severe intra-alveolar and perivascular granulomatous inflammation peaked at day 2 to 3 as evidenced by macroscopic consolidation of pulmonary parenchyma (Figure 2) as well as microscopic granuloma formation (Figure 3). The gross consolidation of the lung subsided by 7 days after i.t. challenge (Figure 2) although focal microscopic granulomas persisted. The granulomas were most often within alveolar spaces that were centered around bronchi and alveolar ducts but were less frequently present in an interstitial or perivenular location. Lymphocytic infiltration was also present within alveolar spaces and the interstitium, most prominently around venules (Figure 3).

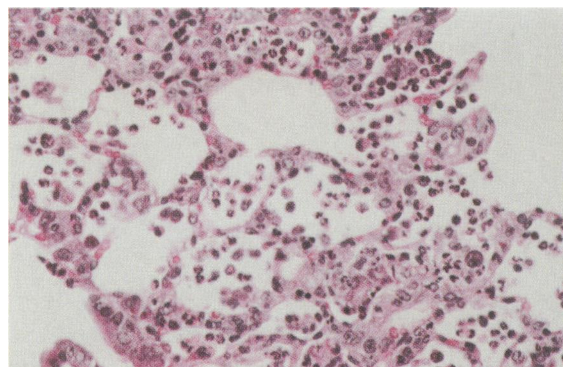

Figure 1. *Neutrophils are the predominant inflammatory leukocytes in the alveolar space 1 day after i.t. challenge with* C. parvum *in sensitized rats.*

The i.v. injection of *C. parvum* 2 weeks after i.v. sensitization caused granulomatous angiitis peaking at 3 days after the i.v. challenge (Figures 4 and 5). Perivascular, interstitial, and occasional intra-alveolar granulomas accompanied the vascular involvement. The conspicuous early neutrophilic inflammation that was seen after i.t. challenge was not observed after i.v. challenge. The granulomatous angiitis was mainly localized at the level of venules although occasional involvement of larger veins and

Figure 2. *A gross photograph of sagittal sections of the right lung inflated with Bouin's fixative shows widespread macroscopically visible nodular consolidation of the lung (surrounded by triangles) 3 days after i.t. challenge with* C. parvum *in sensitized rats. At 1 week after challenge, the macroscopic consolidation has largely subsided.*

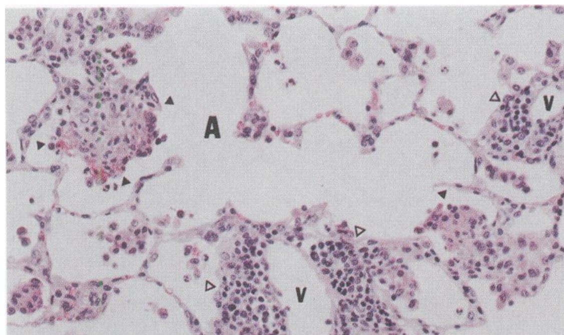

Figure 3. *Several intra-alveolar granulomas composed of epithelioid macrophages are illustrated 3 days after i.t. challenge with* C. parvum *in sensitized rats (A, an alveolar space;* solid arrowheads *point to intra-alveolar granulomas). Note the lymphocytes around venules (V, venules;* open arrowheads *point to cuffs of perivenular lymphocytes).*

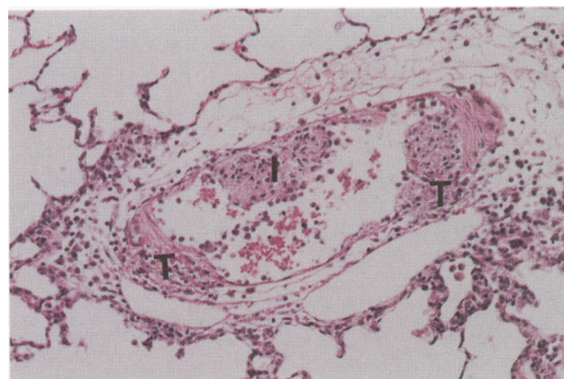

Figure 5. *Granulomatous angiitis in a larger venule shows intimal (I) as well as transmural (T) granulomatous inflammation.*

arterioles was also found. The vascular intima was the most frequent site of vascular inflammation, followed by medial and transmural involvement. The macroscopic appearance of the lungs after i.v. challenge with *C. parvum* was characterized by a fine reticular pattern (Figure 6) suggestive of predominantly interstitial involvement and differing from the nodular consolidation seen after i.t. challenge.

In the lungs of rats that received an i.v. injection of *C. parvum* without subsequent i.t. or i.v. challenge, pulmonary histology at 2 weeks showed mild focal perivascular inflammation with occasional intra-alveolar and intimal granulomas.

Inflammatory Cells in BAL Fluid

The absolute number of leukocytes and neutrophils in BAL fluid peaked 1 day after i.t. injection of *C. parvum* (P = 0.008, at day 1 as compared with control rats; Figure 7). The absolute lymphocyte count in BAL fluid peaked 3 days after the i.t. challenge ($P < 0.0001$, at day 3 as compared with control rats; Figure 7). The number of macrophages and monocytes recovered in BAL fluid was significantly decreased as compared with saline-injected control animals at day 1 and day 3 (P = 0.004 and 0.002, respectively), an observation that may be attributable to the aggregation and increased adherence of these cells (Figure 7).

The bronchoalveolar neutrophil and lymphocyte counts after i.v. challenge peaked at days 3 to 7 (Figure 8). The number of neutrophils recoverable after i.v.-challenge was 10-fold lower than after i.t. challenge (Figure 8).

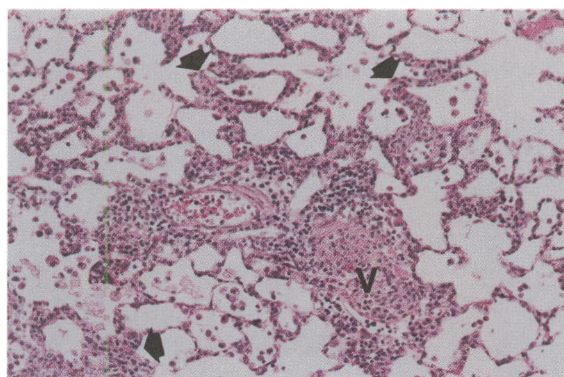

Figure 4. *An i.v. challenge with* C. parvum *in sensitized rats causes granulomatous angiitis and interstitial inflammation in the lung. Note the occlusion of the lumen of a venule by a granuloma (V). Also note the perivenular cuffing of lymphocytes. The granulomatous and lymphocytic inflammatory response meanders along the alveolar walls* (solid arrows) *and provides the histological basis for the grossly observed diffuse reticular thickening of the lung (see Figure 6) as compared with the nodular consolidation (see Figure 2) of the lung after i.t. challenge.*

Cytokine Expression

TNF and IL-6 protein levels in BAL fluid increased after i.t. challenge with *C. parvum*. The kinetics of TNF and IL-6 protein expression in BAL fluid paral-

Figure 6. *A delicate diffuse reticular thickening of the pulmonary parenchyma demonstrates the gross appearance of the lung 3 days after i.v. challenge in a* C. parvum*-sensitized rat. The bronchocentric pattern of nodular consolidation seen after i.t. challenge is shown for comparison and is seen best in the right middle lobe* (arrow).

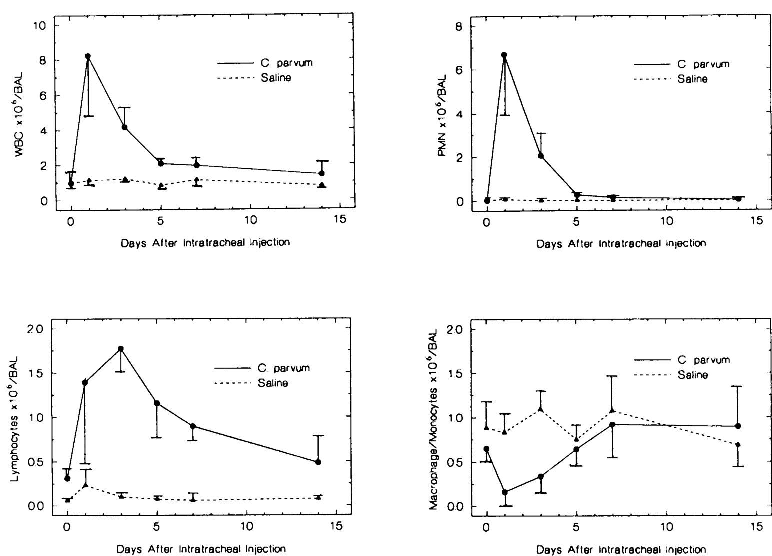

Figure 7. *After i.t. challenge with* C. parvum *in sensitized rats, the number of neutrophils in BAL fluid peaks at 1 day. The number of lymphocytes in BAL fluid peaks at 3 days. A transient decrease in the number of macrophages and monocytes recoverable in BAL fluid is noted as compared with controls, suggesting adherence and aggregation of these cells within the alveolar space as they contribute to granuloma formation.*

leled the exudation of neutrophils into BAL fluid (Figure 9).

The TNF protein level in BAL fluid peaked at 2 days after i.v. challenge but at a much lower concentration than after i.t. challenge (Figure 10). The peak IL-6 levels after i.v. challenge, on the other hand, were comparable to the IL-6 protein levels after i.t. challenge (Figure 10).

Chemokine Expression

Whole-lung RNAse protection assay demonstrated up-regulation of steady-state mRNA expression of

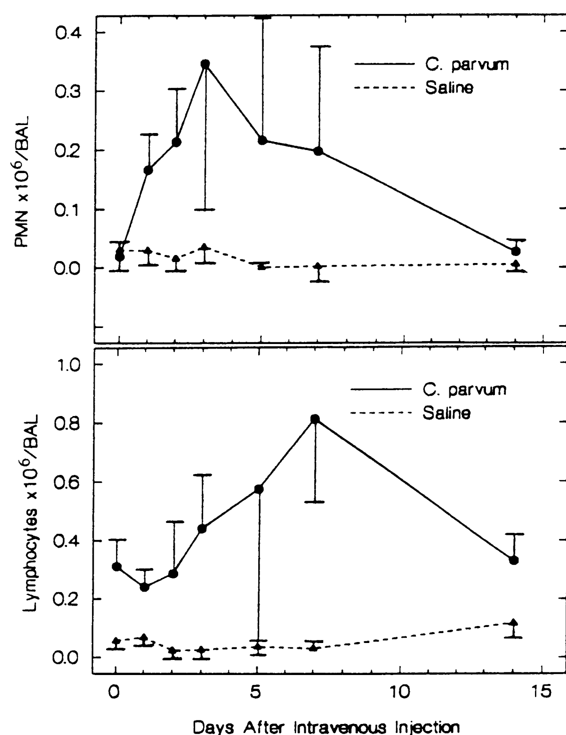

Figure 8. *After i.v. challenge with* C. parvum *in sensitized rats, the number of neutrophils and lymphocytes recovered in BAL fluid is much lower than after i.t. challenge, reflecting the predominantly intravascular and interstitial localization of inflammation after i.v. challenge.*

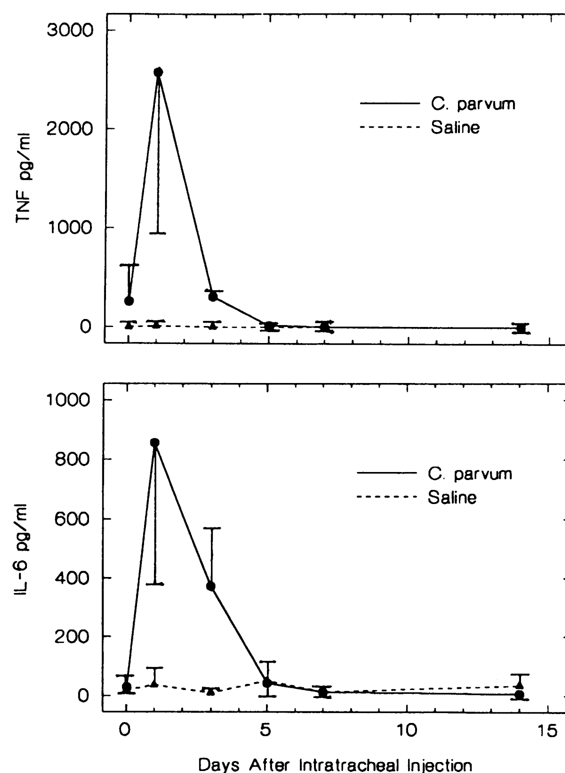

Figure 9. *After i.t. injection of* C. parvum *in sensitized rats, TNF and IL-6 bioactivity in BAL fluid peaks at 1 day and returns to control levels by 5 days.*

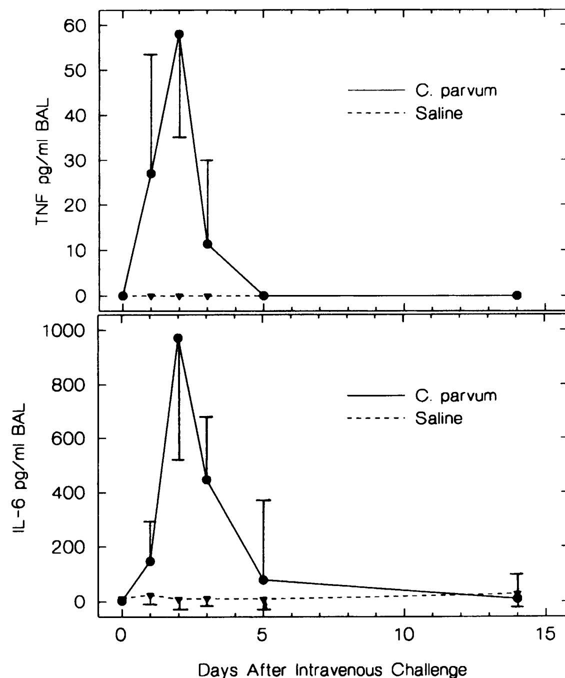

Figure 10. *After i.v. injection of* C. parvum *in sensitized rats, the increase in TNF protein in BAL fluid is less than after i.t. challenge. TNF levels in BAL fluid thus reflect the relative magnitude of neutrophil accumulation within the bronchoalveolar space. On the other hand, IL-6 protein levels in BAL fluid are comparable in i.v.- and i.t.-challenged rats.*

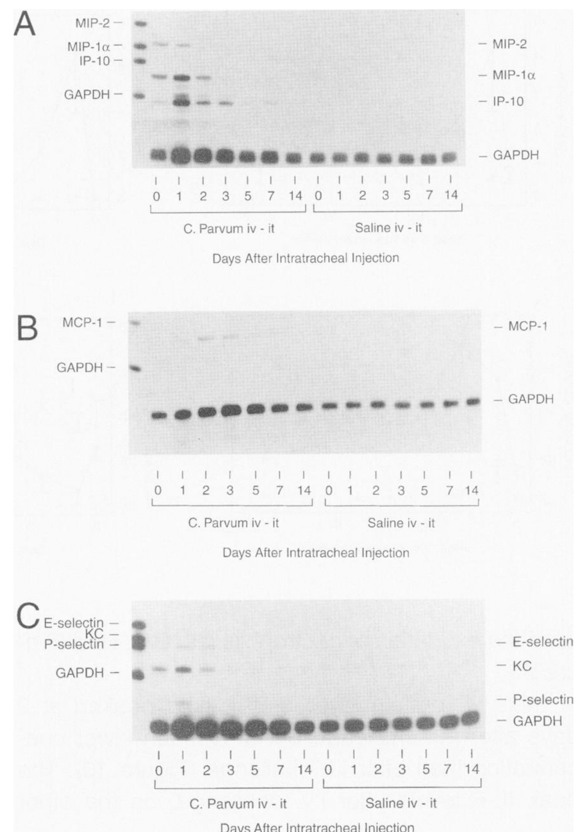

Figure 11. *As demonstrated by RNAse protection assay, CC chemokine (MCP-1 and MIP-1α), CXC chemokine (MIP-2, KC, and IP-10), and adhesion molecule (E- and P-selectin) mRNAs are expressed in whole lung after i.t. challenge with* C. parvum *in sensitized rats. The lungs at day 0 were harvested 30 minutes after i.t. challenge with* C. parvum.

CC (MCP-1 and MIP-1α) and CXC (MIP-2, KC, and IP-10) chemokines after i.t. challenge with *C. parvum* (Figure 11, A–C). The increase in chemokine expression occurred predominantly at 1 to 3 days after the i.t. challenge with *C. parvum* and thus correlated with the peak of inflammation as documented by histology and cell counts in BAL fluid.

Rats challenged with i.v. *C. parvum* revealed mRNA up-regulation of CC chemokines (MCP-1 and MIP-1α) between 1 and 7 days after challenge as compared with controls (Figure 12, A and B). Up-regulation of neutrophil chemotactic CXC chemokines (KC and MIP-2) was less prominent than after i.t. challenge, correlating with the relative paucity of neutrophilic inflammation. Indeed, MIP-2 mRNA was not detected at any of the time points examined after i.v. challenge, and KC expression appeared less than after i.t. challenge. On the other hand, expression of IP-10 was as marked as in i.t.-challenged rats.

Leukocyte and Endothelial Cell Adhesion Molecule Expression

ICAM-1 expression in the lungs of control rats (Figure 13A) was demonstrated immunohistochemically on alveolar epithelial cells but not, or only faintly, on alveolar macrophages, as is consistent with previous immunohistochemical and immunoelectron microscopic studies.[20,21] After i.t. challenge of *C. parvum*, widespread immunoreactive ICAM-1 was found at the cell surface of intra-alveolar and interstitial granuloma-forming histiocytes in the lung (Figure 13B). CD44 immunostaining is detected on the cytoplasmic membranes of macrophages in both control and *C. parvum*-challenged lungs. The intensity of CD44 immunoreactivity appeared to be somewhat greater in the granulomas of *C. parvum*-challenged rats than on the alveolar macrophages of control rats (Figure 14). E-selectin and P-selectin mRNA expression was noted by whole-lung RNAse protection assay at days 1 and 2 after i.t. challenge (Figure 11C).

The granuloma-forming histiocytes in i.v.-challenged rats also demonstrated immunoreactive ICAM-1 and CD44 expression. Alveolar macrophages in i.v.-challenged rats showed little or no ICAM-1 expression. Whole-lung RNAse protection assay of P-selectin and E-selectin showed mRNA

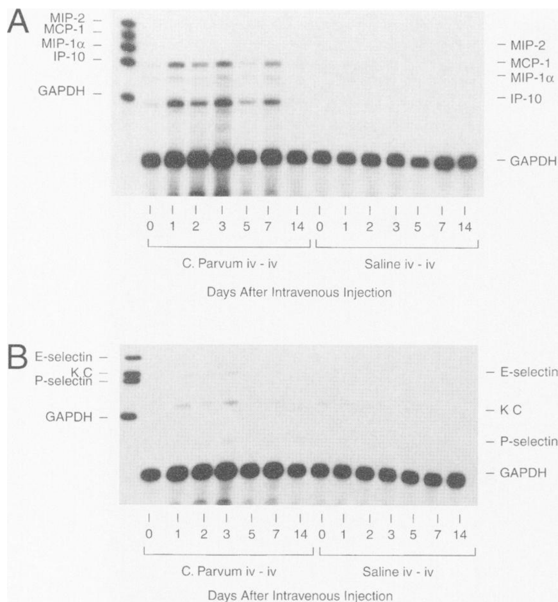

Figure 12. *As demonstrated by RNAse protection assay, C-C chemokine (MCP-1 and MIP-1α), CXC chemokine (KC and IP-10), and adhesion molecule (E- and P-selectin) mRNAs are expressed in whole lung after i.v. challenge with* C. parvum *in sensitized rats.*

up-regulation peaking at days 1 to 3 after i.v. challenge (Figure 12B).

Discussion

The source of antigenic challenge was shown to be an important factor in the pathogenesis of pulmonary granulomatous inflammatory disease. An i.t. antigenic challenge in sensitized rats reached the lungs via the airways and caused a predominantly intraalveolar lesion similar to EAA, a disease caused in patients by repeated respiratory exposure to organic antigens. An i.v. antigenic challenge reaching the lungs via the circulation caused a predominantly angiocentric lesion similar to angiocentric sarcoidosis. The nature and the source of antigenic challenge in patients with sarcoidosis remains unknown. The 2-week interval between sensitization and challenge with *C. parvum* was chosen to allow for the establishment of a cell-mediated immune response. The kinetics of the granulomatous response that occurred at 2 to 3 days was consistent with the time course of delayed-type hypersensitivity reactions such as the tuberculin reaction in humans.

C. parvum has been reported to activate macrophages to produce TNF-α *in vitro*,[22] and TNF has previously been implicated by Chensue and colleagues[23–25] in the pathogenesis of pulmonary granuloma formation. A role for TNF-α in granulomatous inflammation has been demonstrated in *Schistosoma mansoni* egg-induced granulomatous inflammation in rats and in SCID mice.[25,26] A larger increase in TNF protein in BAL fluid was observed after i.t. than after i.v. challenge with *C. parvum*. A greater accumulation of neutrophils in BAL fluids was also noted after i.t. than after i.v. challenge. The neutrophilic inflammation that preceded the peak of granuloma-

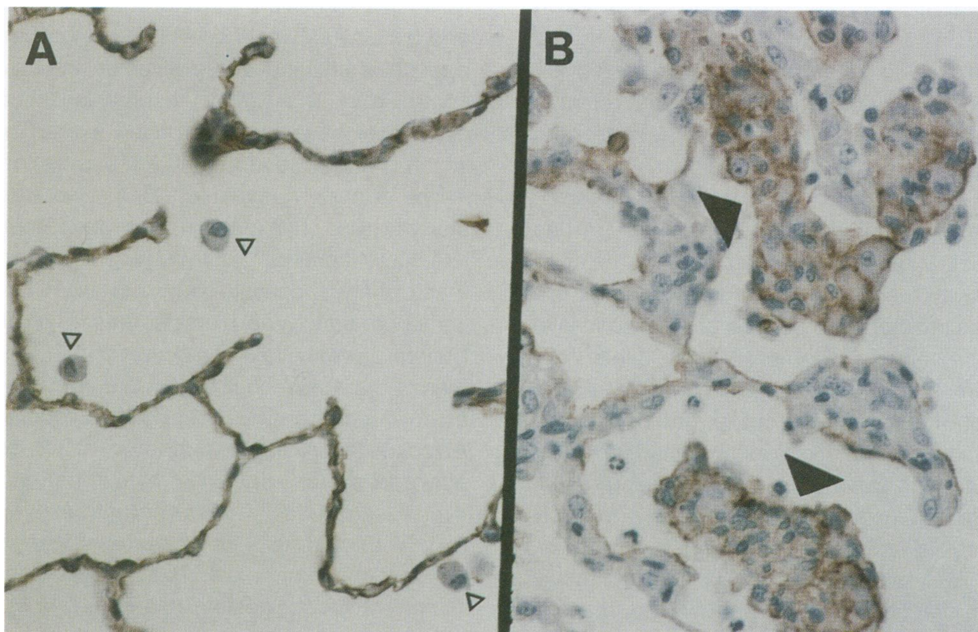

Figure 13. *ICAM-1 immunoreactivity is present along the alveolar epithelium in both saline-injected control rats* (A) *and i.t.* C. parvum*-challenged rats* (B). *The alveolar macrophages in control rats show little or no immunoreactivity for ICAM-1* (open arrowheads). *The macrophages within the granulomas of* C. parvum*-treated rats demonstrate strong ICAM-1 immunoreactivity* (solid arrowheads).

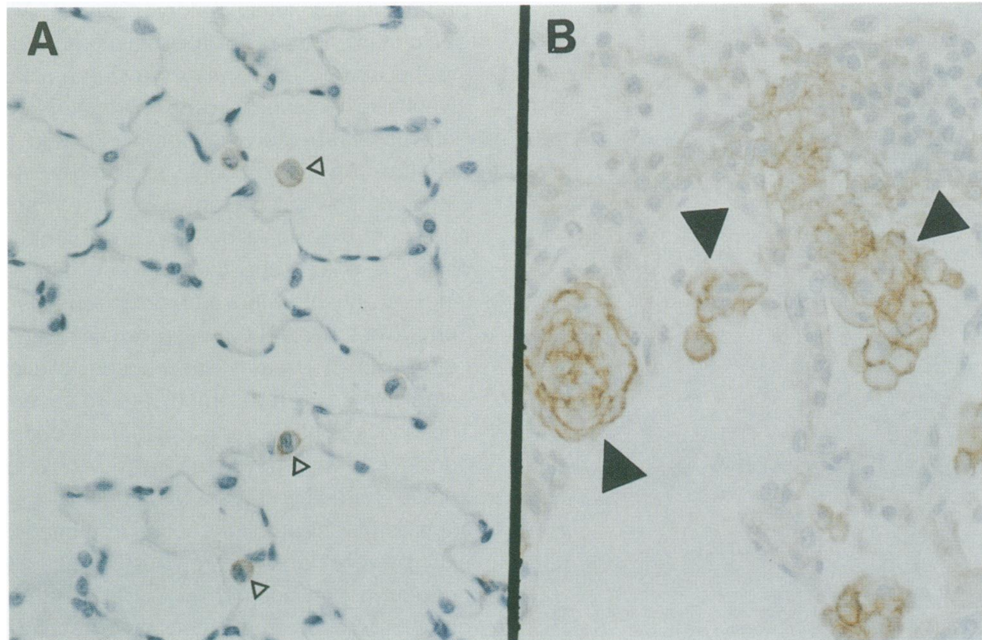

Figure 14. *CD44 immunoreactivity is present in the alveolar macrophages of control rats* (A, open arrowheads) *as well as along the cell membranes of macrophages in granulomas* (B, solid arrowheads). *The intensity of CD44 immunoreactivity appears greater in the epithelioid macrophages of granulomas.*

tous inflammation after i.t. challenge coincided with the peak of TNF expression, suggesting a role for soluble TNF in the recruitment of these neutrophils as well as in granuloma formation. The BAL IL-6 protein level in i.v.-challenged rats, however, was not significantly different than the IL-6 levels after i.t. challenge.

The participation of CXC (MIP-2, KC, and IP-10) and CC (MIP-1α and MCP-1) chemokines in the pathogenesis of inflammation was suggested by mRNA expression as compared with control rats. The i.v. challenge with *C. parvum*, like i.t. challenge, caused mRNA expression of the CXC chemokine IP-10. IP-10, an interferon-γ-inducible CXC chemokine lacking neutrophil chemotactic activity,[27,28] has been localized immunohistochemically in delayed hypersensitivity reactions elicited in the skin of leprosy patients injected with purified protein derivative from mycobacterium.[29] Up-regulation of other neutrophil chemotactic CXC chemokines (MIP-2 and KC) was not prominent in i.v.-challenged rats, correlating with the paucity of neutrophilic inflammation. In a rat model of glucan-induced pulmonary granulomatous angiitis, Warren and colleagues[30] previously showed the role of MCP-1 by demonstrating an increase in mRNA expression in whole lung and by the neutralizing effect of anti-MCP-1 antibody. Lukacs et al[31] have reported that MIP-1α contributes to cellular recruitment during *S. mansoni* egg-induced granulomatous inflammation. MIP-1α expression is increased in the BAL fluid and alveolar macrophages of patients with hypersensitivity pneumonitis.[32]

The importance of the adhesion molecule ICAM-1 has been documented in granulomatous as well as in acute pulmonary inflammation.[25,33] ICAM-1 is constitutively expressed by alveolar epithelium.[33] Immunohistochemical staining suggested the induction of ICAM-1 expression in aggregated macrophages and histiocytes within granulomas in contrast to the paucity of staining in the alveolar macrophages in control rats. The ICAM-1 expression in the histiocytes may have been mediated in part by TNF-α, which is known to induce ICAM-expression.[25]

The adhesion molecule CD44, a receptor for hyaluronic acid (hyaluronan), has been implicated in leukocyte homing.[34] Although pulmonary alveolar macrophages constitutively express CD44, CD44 immunoreactivity appears increased on the membranes of epithelioid histiocytes within the granulomas of *C. parvum*-challenged rats as compared with alveolar macrophages in saline-injected control rats. Alveolar macrophages undergo CD44-dependent aggregation by addition of hyaluronan *in vitro*.[34] A clinical study has shown an increase in hyaluronan in BAL fluid in farmer's lung (a form of EAA) but not in asymptomatic farmers.[35] The possible role of hyaluronan in EAA suggests a role for CD44 in the intra-alveolar granulomatous response of EAA.

The expression of adhesion molecules in the selectin family, E-selectin and P-selectin, appeared to

be increased in the *C-parvum*-challenged lungs in both models. E-selectin and P-selectin have both been previously implicated in the recruitment of neutrophils to local sites of acute inflammation including the lung.[36,37]

In conclusion, i.t. or i.v. *C. parvum* challenge to rats immunized with *C. parvum* causes pulmonary granulomatous inflammation simulating EAA or angiitic pulmonary sarcoidosis, suggesting the route of antigenic challenge as a pathogenetic factor in these granulomatous lung diseases in humans. The increased inflammatory activity in the air space and BAL fluid after i.t. challenge (as compared with i.v. challenge) is most likely related to the intra-alveolar expression of alveolar-macrophage-derived proinflammatory mediators. The histopathology noted after i.v. challenge with *C. parvum* suggests that angiitic sarcoidosis may be pathogenetically related to the vascular dissemination of an unknown antigen in sensitized patients. The described models may be useful to study the pathophysiological roles of cytokines, chemokines, and adhesion molecules in EAA and pulmonary angiitic sarcoidosis.

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