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Cytokines in *Mycoplasma hyorhinis*-Induced Arthritis in Pigs Bred Selectively for High and Low Immune Responses

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Yorkshire pigs were bred selectively for high and low immune responses (H and L pigs, respectively) based on multiple antibody (Ab) and cell-mediated immune response traits. In a previous experiment, generation 4 (G4) pigs of each line were infected with *Mycoplasma hyorhinis*. High responders had a more rapid and higher Ab response and less polyserositis, but arthritis was more severe in H pigs than in L pigs. To test the hypothesis that line differences were attributable to differential expression of cytokines, *M. hyorhinis* infection was induced in pigs of G8. Arthritis was more severe clinically (P, ≤0.05) and postmortem (P, ≤0.001) when *M. hyorhinis* CFU were more numerous in synovial fluid (SF) of H pigs than of L pigs (P, ≤0.03). In H pigs but not L pigs, CFU and lesion scores were correlated positively. In H pigs, infection increased the frequency of expression of mRNAs for interleukin-8 (IL-8), IL-10, and tumor necrosis factor alpha (TNF-α) in mononuclear cells from synovial membranes (SM). In L pigs, IL-1α, IL-6, IL-10, and TNF-α mRNAs were increased in frequency of expression. The quantity of the cytokine message for IL-6 was increased in infected H pigs. For L pigs, infection increased the cytokine message for IL-1α, IL-6, IL-10, and TNF-α. IL-6 in SM and gamma interferon (IFN-γ) in SF were produced at a higher copy number in H pigs than in L pigs after infection. For H pigs, there were no positive rank correlations between lesion or CFU scores and cytokines. For L pigs, IL-1α, IL-8, IL-10, and TNF-α in SM correlated with CFU, while IL-6, TNF-β, and IFN-γ in SF correlated with CFU. Lesion score in L pigs correlated with IL-1α in SF. While these results indicate that H and L pigs differ in the cytokine response to *M. hyorhinis* infection, they do not confirm a characteristic cytokine response in association with the relative susceptibility to infection and arthritis observed in H pigs.

Mice selected for a high antibody (Ab) response to sheep erythrocytes without concurrent selection for a cell-mediated immune (CMI) response (3) have increased resistance to organisms for which Ab is the principal resistance mediator but are not more resistant to intracellular pathogens for which CMI is a critical resistance determinant (4, 9). Yorkshire pigs were bred for high and low immune responses (H and L pigs, respectively) based on simultaneous selection for Ab and CMI-related traits (22). Selection was based on an index that combined estimated breeding values for serum immunoglobulin G concentration, Ab response to hen egg white lysozyme, in vitro lymphoproliferative response to concanavalin A, and cutaneous delayed-type hypersensitivity induced by intradermal injection of purified protein derivative of tuberculin after sensitization with bacillus Calmette-Guérin (22). The selection was undertaken to evaluate the effect of high and low Ab and CMI responses on health and productivity.

H pigs produce more Ab to most antigens, including those of complex organisms such as *Actinobacillus pleuropneumoniae* (19), leptospiras, and influenza virus given as vaccines (33). H pigs produce Ab of higher avidity than L pigs (1). H pigs also gain weight more rapidly and tend to have larger litters than control (unselected) or L pigs (21). At generation 4 (G4) of selection, H pigs infected with *Mycoplasma hyorhinis* produced Ab more quickly and to a higher titer than L pigs, had less severe serositis, but developed more severe arthritis (20). These results suggest fundamental differences between the se-

lected lines in immunological homeostasis and inflammation, possibly mediated by alterations in the balance of regulatory and inflammatory cytokines. To further investigate these putative differences, cytokine mRNA expression was studied by quantitative reverse transcription (RT)-PCR with H and L pigs of G8 after infection with *M. hyorhinis*.

MATERIALS AND METHODS

Design. The experiments were done with G8 Yorkshire H and L pigs in a split-litter design, as was previously used to study responses to *M. hyorhinis* (20). Pairs of littermates were of either sex and 40 to 76 days old. Each pair of littermates included *M. hyorhinis*-infected and uninfected piglets. In total, there were 10 pairs of H pigs and 11 pairs of L pigs. All experiments were conducted with approval of the local animal care committee according to guidelines of the Canadian Council for Animal Care.

Infection with *M. hyorhinis*. *Mycoplasma hyorhinis* strain 497-14, originally isolated from an arthritic joint of a naturally infected pig, was grown in modified Hayflick's broth (10), washed, suspended in phosphate-buffered saline (PBS), and stored at −70°C (20). For infection, 2×10^9 *M. hyorhinis* CFU in 2 ml of PBS was injected intraperitoneally, while the paired littermate received 2 ml of PBS.

Antemortem and postmortem observations. Pigs were monitored for 14 days after infection and scored daily for signs of arthritis. A score of 0 was assigned when no signs were observed. A score of 1 was associated with one or more slightly swollen joints. A score of 2 was assigned when two or more joints were moderately swollen and the animal was lame and reluctant to move. The maximum score of 3 was used when two or more joints were severely swollen in association with severe lameness and reluctance to move. Pigs given a score of 3 were euthanatized and assigned a score of 3 for each remaining day of the experiment to day 14. All other pigs were killed on day 14 with intravenous barbiturates. Total accumulated scores for each pig were used to describe and analyze antemortem responses to infection. Postmortem examinations were conducted on all pigs, and lesion scores were assigned as previously described (20). For arthritis, scores were derived as follows: no lesions, 0; slight or moderate hyperemia or edema of joint synovial membranes (SM) with moderately increased synovial fluid (SF) in one or more joints, 1; moderately increased, turbid SF with moderate edematous villous hypertrophy of SM in one or more joints, 2; and highly increased, very turbid SF with very edematous hypertrophy of SM in one or more joints, 3. All assessments were macroscopic and confirmed by three independent observers without knowledge of the infection or selection line status of the subject.

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Samples and preparation of cells. Blood was collected into heparinized containers from the retrobulbar sinus on days −1, 0, 4, 7, 11, and 14. Blood and SF from arthritic joints were collected postmortem, and 10 μl of each was inoculated into modified Hayflick's agar and incubated for 8 days at 37°C. *M. hyorhinis* CFU were scored as follows: no colonies, 0; 1 to 10, 1; 11 to 100, 2; 101 to 1,000, 3; and >1,001, 4. Scores for individual joints were summed to obtain the score for any given pig. Serum Ab and SF Ab to *M. hyorhinis* were determined by indirect hemagglutination (7). Differential leukocyte counts in SF samples were obtained by routine procedures.

From infected pigs, single-cell suspensions were prepared from SF and SM of arthritic joints. From uninfected controls, only SM were used, since SF could not be reliably obtained from normal joints. Mononuclear cells (MNCs) from SF were obtained by Ficoll-Hypaque (specific gravity, 1.072; Sigma Chemical Co.) density gradient centrifugation.

SM were processed by enzyme digestion (6) to obtain single-cell suspensions. Thinly sliced SM were incubated at 37°C for 2 h in complete medium (RPMI 1640; Gibco BRL, Burlington, Ontario, Canada) plus 5% fetal calf serum (Sigma) and containing 5 mg of collagenase type IV (Sigma) per ml and 150 μg of DNase type I (Sigma) per ml. Tissue suspensions were filtered through sterile gauze and through 200 μm-pore-size nylon fabric (Nitex; B and SH Thompson, Scarborough, Ontario, Canada) into a sterile container. Cells were washed three times with RPMI 1640 and resuspended in the same medium.

RNA extraction. Total RNA was extracted from MNCs obtained from SF and SM by the acid guanidinium thiocyanate method (8), and the nucleic acid concentration was determined by measuring the optical density at 260 nm (GeneQuant II; Pharmacia Biotech Inc., Baie d'Urfe, Quebec, Canada). All samples were treated with amplification-grade DNase I (Gibco BRL) to eliminate genomic DNA contamination.

Cytokine mRNA analysis. Cytokine mRNA expression was evaluated by quantitative RT-PCR with an internal control containing gene-specific primer sequences for interleukin-1α (IL-1α), IL-2, IL-4, IL-6, IL-8, IL-10, tumor necrosis factor alpha (TNF-α), TNF-β, gamma interferon (IFN-γ), and β2-microglobulin (β2-m) (25). The method used was based on that of Wang et al. (32) but was modified by including the β2-m gene as a "housekeeping gene" to control for the integrity of sample nucleic acids.

cDNA synthesis. The plasmid construct was linearized with *Eco*RI, and control RNA was obtained by transcription at 37 to 40°C for 2 h with SP6 polymerase (Riboprobe II core system; Promega Corporation, Madison, Wis.). RT was carried out with a 10- to 60-μl reaction mixture (procedure A) containing 50 ng to 5 μg of total RNA and 4.3×10^6 to 8.6×10^6 molecules of control RNA or a 20-μl reaction mixture (procedure B) containing 250 to 500 ng of total RNA and 8.6×10^3 molecules of control RNA. Procedure A was used for the evaluation of both cytokine and β2-m messages. Since β2-m was expressed at a high copy number, the message for β2-m was quantified using a larger number of starting control RNA molecules. In contrast, the copy number for IL-2 was low, so quantification was carried out using a smaller number of control RNA molecules, according to procedure B. The reaction mixtures contained 25 mM $MgCl_2$, PCR buffer II (50 mM KCl, 10 mM Tris-HCl [pH 8.3]), 1 mM each deoxynucleoside triphosphate, (dNTP), 1 U of RNase inhibitor per μl, 2.5 U of murine leukemia virus reverse transcriptase per μl, and 2.5 μM random hexamers (GeneAmp RNA PCR kit; Perkin-Elmer, Mississauga, Ontario, Canada). Tubes were incubated sequentially for 10 min at room temperature, 60 min at 42°C, 8 min at 99°C, and 5 min at 5°C using a thermal cycler (RoboCycler Gradient 96; Stratagene-PDI Bioscience, Aurora, Ontario, Canada).

PCR. Five microliters of cDNA was diluted serially in 1:3 increments, and amplifications were performed by "hot-start PCR" for 33 to 38 cycles in the thermal cycler. Each cycle involved denaturation (94°C for 1 min), annealing (55°C for 1 min), extension (72°C for 1 min), and a final extension (72°C for 10 min). The PCR was carried out with a 25-μl reaction mixture consisting of PCR buffer II, 1.5 mM $MgCl_2$, 200 μM each deoxynucleoside triphosphate, 0.12 μM each sequence-specific primer, and 0.625 U of *Taq* DNA polymerase (Gibco BRL). Products of PCR were separated by 2% agarose gel electrophoresis and stained with ethidium bromide. Amplified products for IL-2 were resolved in 7.5% polyacrylamide gels. Gels were photographed with 667 Polaroid film by use of a UV transilluminator, and images of gels were scanned and analyzed densitometrically (Molecular Analyst; BioRad Laboratories, Mississauga, Ontario, Canada). Data were converted to $\log_{10}$ density, and target mRNA copy number was calculated using control curves and extrapolated to 1 μg of total RNA as described elsewhere (25a, 32).

Statistical analysis. Statistical analysis was performed with GraphPad Prism version 3.0 (GraphPad Software, San Diego, Calif.). Clinical scores, postmortem scores, and CFU of *M. hyorhinis* were compared between lines using a one-tailed Student *t* test. Serum and SF log Ab titers within and between infected and control pigs were compared using a one-tailed Student *t* test. All samples were analyzed for β2-m and cytokine mRNA expression. Values for β2-m were compared by sample type between lines using the *t* test to determine if expression varied. As differences were not significant, β2-m values were averaged for both lines to obtain an expected value to which each cytokine mRNA value was normalized as follows: expected value for β2-m/observed value for β2-m = *x*. The expected value for the target as copies per microgram of total RNA was then calculated by multiplying *x* by the observed copy number for the target. Three approaches were taken to data analysis. (i) Cytokine mRNA expression frequency was compared between lines and treatments using Fisher's exact test. (ii) Quantity of cytokine mRNA was compared between lines and treatments by the Mann-Whitney test adjusted for ties. (iii) Rank correlations were made by Pearson analysis between postmortem lesion scores, *M. hyorhinis* CFU scores, and cytokine mRNAs (copies per microgram of total RNA). Significant differences are reported at a *P* value of ≤0.05, and trends are reported at a *P* value of ≤0.1. Where appropriate, Bonferroni-Sidak (BS)-adjusted probabilities (29) are provided together with the unadjusted exact values of *P*.

RESULTS

All *M. hyorhinis*-infected but not uninfected pigs developed clinical disease, predominantly arthritis, with earlier onset and greater severity in H pigs than in L pigs (Fig. 1). Clinical arthritis scores for H pigs and L pigs were 17.1 ± 10.3 and 10.7 ± 6.9 (*P*, ≤0.05), respectively. Five H pigs were killed on day 6 postinfection because of the severity of arthritis. For arthritis at necropsy, scores were 2.4 ± 0.7 and 1.2 ± 0.8 (*P*, ≤0.001) (Fig. 1) for H pigs and L pigs, respectively. *M. hyorhinis* CFU scores in SF of H pigs and L pigs were 4.4 ± 2.1 and 2.2 ± 2.6 (*P*, ≤0.03), respectively (Fig. 1). The correlation between lesion and CFU scores was not significant for L pigs (Pearson's r^2, 0.02; *P*, ≤0.36) but was significant for H pigs (r^2, 0.42; *P*, ≤0.02). Cells within SF of arthritic joints were 78% neutrophils and 22% mononuclear cells (lymphocytes, macrophages, and synoviocytes). Peritonitis, pericarditis, and pleuritis did not differ in severity by line. Serum Ab titers to *M. hyorhinis* were significantly higher in infected pigs than in uninfected pigs (*P*, ≤0.05). Ab titers did not differ by line except on day 0, when values for H pigs were higher than those for L pigs (*P*, ≤0.05). Ab titers were higher in SF than in serum (*P*, ≤0.001) and did not differ by line.

In uninfected pigs, IL-1α, IL-6, and TNF-α mRNAs were detected in SM of H and L pigs, while IL-10 was also present in L pigs. The number of pigs expressing IL-1α was greater for uninfected H pigs than for uninfected L pigs (*P*, ≤0.06) (Fig. 2A). Infection with *M. hyorhinis* altered the frequency of pigs that expressed mRNA for several cytokines in SM (Fig. 2B and C). Uninfected and infected H and L pigs differed in cytokine mRNA expression. In H pigs, IL-8 (*P*, 0.09), IL-10 (*P*, 0.0001), and TNF-α (*P*, 0.02) increased in frequency of expression, while in L pigs, IL-1α (*P*, 0.003), IL-6 (*P*, 0.02), IL-10 (*P*, 0.001), and TNF-α (*P*, 0.006) increased. Messages for IL-2 and IL-4 were absent from all pigs. Breeding lines (H and L pigs) did not differ significantly in frequency (Fisher's exact test) of expression of cytokine mRNA postinfection.

Quantities of cytokine mRNA in SM were compared for infected and uninfected pigs of each line, and the significance of differences was tested by the Mann-Whitney method (Fig. 3). There were no differences between lines for any cytokine in uninfected pigs. In infected H pigs, mRNA for IL-6 was increased (*P*, ≤0.09) (Fig. 3A). In addition, IL-8 and IL-10 mRNAs were detected only in infected pigs (Fisher's exact test: *P* for IL-8, 0.09; *P* for IL-10, 0.0001). Infected L pigs had more IL-1α (*P*, ≤0.08), IL-6 (*P*, ≤0.09), IL-10 (*P*, ≤0.01), and TNF-α (*P*, ≤0.03) than uninfected L pigs (Fig. 3B). Infected H and L pigs did not differ in quantities of cytokine mRNAs except for IL-6, which was higher in SM of H pigs (*P*, ≤0.09) (Fig. 3C), and IFN-γ, which was higher in SF of H pigs (*P*, ≤0.08) (Fig. 3D).

Lesion and CFU scores were correlated by Pearson's analysis with cytokine mRNAs (copies per microgram of total RNA) in SM and SF of *M. hyorhinis*-infected H and L pigs. There were no significant correlations in H pigs. In SM of L pigs, CFU scores correlated positively with mRNAs of IL-1α (r^2, 0.61; *P*, 0.008; BS value, 0.0471), IL-6 (r^2, 0.63; *P*, 0.006; BS value, 0.0355), IL-8 (r^2, 0.83; *P*, 0.0002; BS value, 0.0012), IL-10
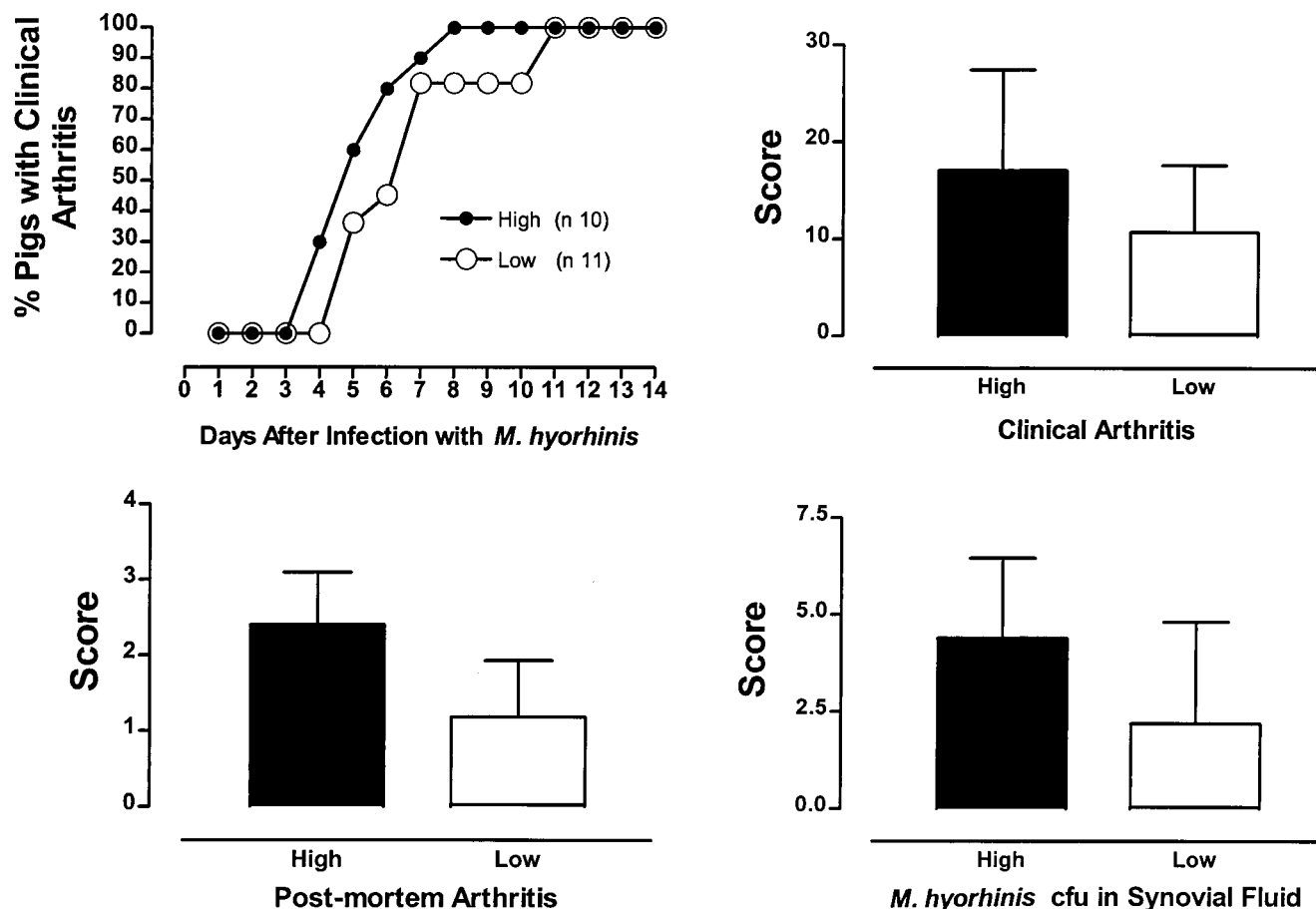
FIG. 1. Responses of H and L pigs to experimental infection with *M. hyorhinis*. Scores are based upon observed arthritis and CFU of *M. hyorhinis* in SF according to criteria described in the text. Scores for individual pigs are represented by closed and open circles for H and L pigs, respectively. Means and standard deviations are indicated by bars. All comparisons of scores between lines were significant (*P*, ≤0.05).

(r^2, 0.51; *P*, 0.02; BS value, 0.1114), and TNF-α (r^2, 0.65; *P*, 0.005; BS value, 0.0279). In SF of L pigs, CFU scores correlated positively with mRNAs of IL-6 (r^2, 0.98; *P*, 0.0001; BS value, 0.0007)), TNF-β (r^2, 0.61; *P*, 0.01; BS value, 0.0850), and IFN-γ (r^2, 0.72; *P*, 0.004; BS value, 0.0249). Lesion score was positively correlated only with mRNA of IL-1α (r^2, 0.50; *P*, 0.0329; BS value, 0.2088) in SF of L pigs. BS-corrected probabilities for comparisons involving six cytokines (SM in L pigs) or seven cytokines (SF in L pigs) are indicated above following the exact *P* values. BS-adjusted values for 90, 95, and 99% confidence intervals involving six comparisons were 0.4686, 0.2650 and 0.0585, respectively. For seven comparisons, the equivalent values were 0.5217, 0.3017, and 0.0680.

DISCUSSION

M. hyorhinis infection of pigs induced the anticipated clinical and postmortem signs (20, 26). Resistance to *M. hyorhinis* infection in H and L pigs of G8 varied by line in that arthritis was more severe in H pigs than in L pigs, confirming the results for pigs of G4 (20). However, polyserositis (pleuritis, pericarditis, and peritonitis) did not differ by line in G8 as in G4. Ab titers to *M. hyorhinis* did not vary by line in sera or SF except on day 0, when serum Ab levels were higher in H pigs than in L pigs. In the study of G4 pigs, H pigs had a more rapid onset of a serum Ab response, which

reached higher titers than in L pigs. In the present experiment, five H pigs were killed on day 6 because of severe arthritis, and serum Ab titers of these animals were compared with those on day 7 in L pigs. This procedure may have contributed to the lack of line-related differences in serum Ab titers. Also, differences in immune response traits (Ab plus CMI) between the selected lines were maximum at G4, after which the response to continued selection diminished (21). Hence, line-related differences were expected to be less evident at G8.

The present study was conducted to test the hypothesis that the variation in *M. hyorhinis* infection-related arthritis in H or L pigs is associated with the differential expression of cytokines. A comparison of uninfected and infected H pigs indicated that the frequency of expression of IL-8, IL-10, and TNF-α mRNAs increased after infection with *M. hyorhinis*. In L pigs, the expression of IL-10 and TNF-α mRNAs also increased, as did that of mRNAs for IL-1α and IL-6. Cytokines associated with *M. hyorhinis* infection may reflect immune response and/or inflammatory functions that play a role in the development of arthritis. Messages for IL-2 and IL-4 were not detected in any of the tissues studied. Kita et al. (15) did not detect IL-2 or IL-4 in human MNCs stimulated in vitro with several mycoplasma species, including *M. hyorhinis*, and IL-4 was not detected in SF of patients with chronic juvenile arthritis (17). However, after

A   Uninfected: H and L Pigs
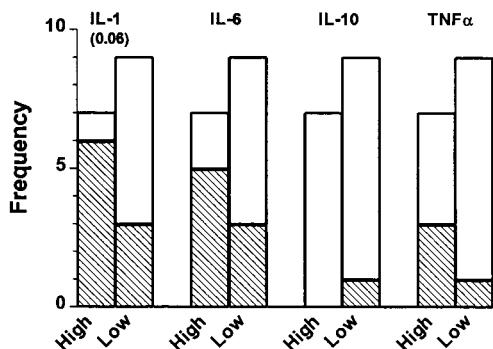
B   Uninfected vs Infected: H Pigs
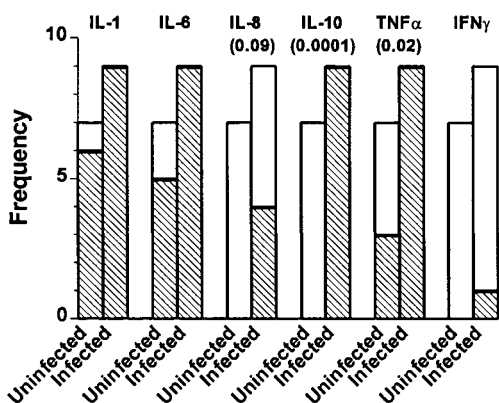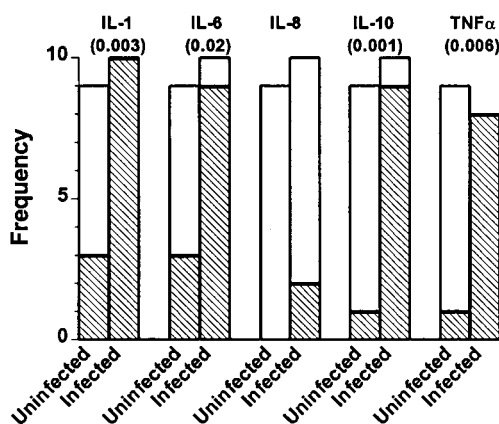

C   Uninfected vs Infected: L Pigs

FIG. 2. Frequency of pigs expressing mRNAs for cytokines. (A) Cytokine mRNA expression in SM of uninfected H ($n = 7$) and L ($n = 9$) pigs. (B) Cytokine mRNA expression in SM of uninfected ($n = 7$) and *M. hyorhinis*-infected ($n = 9$) H pigs. (C) Cytokine mRNA expression in SM of uninfected ($n = 9$) and *M. hyorhinis*-infected ($n = 10$) L pigs. Open and hatched areas of bars represent the frequencies of pigs that were negative and positive, respectively, for cytokine-specific RNA. *P* values resulting from Fisher's exact test are provided in parentheses. *P* values of ≤ 0.05 are taken to be significant, while *P* values of ≤ 0.1 are taken to indicate a trend. *P* values are not provided for comparisons failing to indicate significance or trends.

various stimuli, pigs or pig cells often do not produce IL-2 or IL-4 or produce them only in small amounts (23, 25a, 25b). The apparent lack of increased IL-1α mRNA expression in infected H pigs but not L pigs may reflect the fact that uninfected H pigs more frequently expressed IL-1α mRNA than did L pigs (Fig. 2A). Arthritis was more severe in H pigs than in L pigs, with corresponding line-associated differences in joint fluid *M. hyorhinis* CFU as well as in IL-6 mRNA in SM (Fig. 3C) and IFN-γ mRNA in SF MNCs (Fig. 3D). Increased IL-6 mRNA in SM of infected joints of H pigs may have contributed to arthritogenesis, since IL-6 has been associated with infection-associated inflammation in pigs (23). In adjuvant- and collagen-induced arthritis of mice, IFN-γ is proinflammatory in the initial phase and antiarthritogenic as the disease progresses to remission (5, 14). Since the present study investigated acute infection with *M. hyorhinis*, IFN-γ may have had an arthritogenic role. In human chronic juvenile arthritis, mRNA for IL-6, together with mRNAs for TNF-α and IL-1α, was increased in SF (17), while IL-4 and IL-2 were undetectable. This pattern was assumed to reflect the local prevalence of inflammatory cytokines (17).

Scores for CFU were higher in H pigs than in L pigs and were positively correlated with lesion score only in H pigs, suggesting line-related differences in responses to *M. hyorhinis* infection. A further indication of infection-associated line differences postinfection was obtained from correlations between CFU and lesion scores and cytokine mRNA expression. For CFU scores, positive correlations occurred only in L pigs and involved IL-1α, IL-6, IL-8, and TNF-α in SM as well as IL-6, TNF-β, and IFN-γ in SF. For lesion scores, the only positive correlation was with IL-1α in SF of L pigs. Given that H pigs had more severe arthritis than L pigs, the cytokine response of L pigs may represent a less inflammatory mosaic than that in H pigs. Alternatively, in this study a key cytokine response associated with arthritis in H pigs may not have been among those actually measured.

Mycoplasmas may induce cytokines. For example, *M. hyorhinis* has been shown to induce IL-1α, IL-6, and TNF-α in human monocytes (15, 27), and TNF-α-inducing activity is attributed to acylated proteins (16). The levels of these cytokines were also increased in *M. hyorhinis*-infected pigs in the present study. In addition, the role of TNF-α as well as IL-1α, IL-6, IL-8, and granulocyte-macrophage colony-stimulating factor in the development of rheumatoid or reactive arthritis has been well documented (11, 18). If cytokine production is induced by the multiplication of mycoplasmas, then H pigs might be expected to produce more inflammatory cytokines, including TNF-α, IL-1α, and IL-6, than L pigs, given the higher CFU of *M. hyorhinis* in joints of H pigs. However, only IL-6 and IFN-γ mRNAs were expressed more in joints of H pigs. However, a reduced activity of TNF-α is reported to occur in association with an increased concentration of cytosolic proteins of *M. hyorhinis* (16). Such a phenomenon may explain the lack of line differences in detected cytokine messages. Since *M. hyorhinis* is mitogenic for B cells (24, 30, 31), some cytokine mRNAs may be from B cells that are directly stimulated by *M. hyorhinis*.

As in a previous study (20), CFU of *M. hyorhinis* were higher in joints of H pigs than of L pigs, suggesting a fundamental difference in the interaction between *M. hyorhinis* and members of each line of immune response-selected pigs. In other immune response-based selection experiments, lines have varied in response to bacterial infection. For example, in H mice versus L mice of strain Biozzi, increased growth of *Listeria monocytogenes* in the initial phase of infection was related to
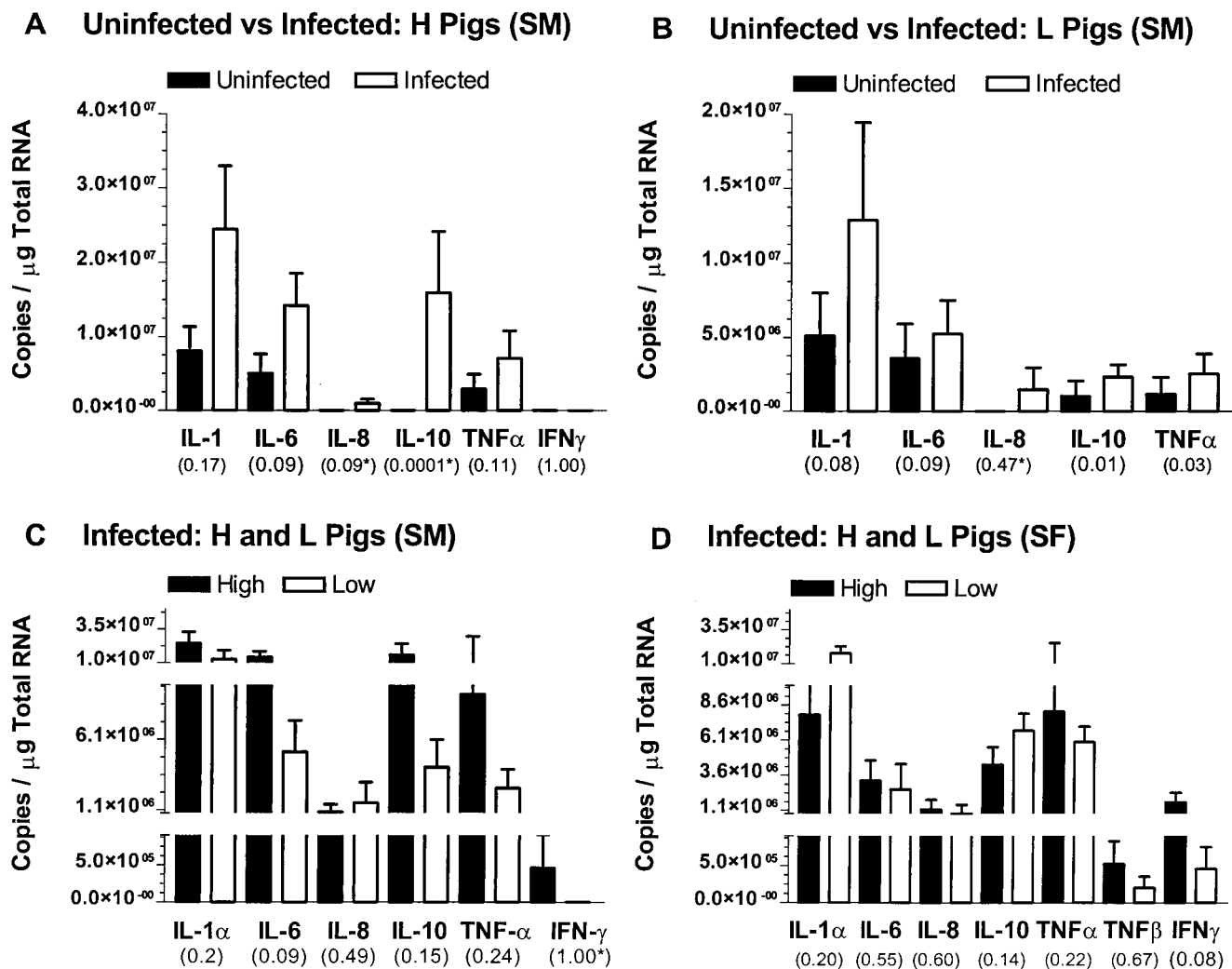
FIG. 3. Cytokine mRNAs (copies per microgram of total RNA) in H and L pigs that were uninfected or infected with *M. hyorhinis*. (A) Cytokine mRNA in SM of uninfected ($n = 7$) and infected ($n = 9$) H pigs. (B) Cytokine mRNA in SM of uninfected ($n = 9$) and infected ($n = 10$) L pigs. (C) Cytokine mRNA in SM of H ($n = 9$) and L ($n = 10$) pigs infected with *M. hyorhinis*. (D) Cytokine mRNA in SF of H ($n = 9$) and L ($n = 9$) pigs infected with *M. hyorhinis*. Means and standard errors are indicated by bars. Probabilities of significant differences for comparisons between groups are given in parentheses and, unless marked by an asterisk, result from the Mann-Whitney test. Values appearing with an asterisk are derived from Fisher's exact test, which was applied when one of the groups compared had no positive values.

differences in macrophage bactericidal activity (2). Although mycoplasmas are not facultative intracellular pathogens, macrophages nevertheless may play a crucial role in mediating resistance to *M. hyorhinis* infection. However, the H and L pigs used here, unlike Biozzi mice, were selected on the basis of combined Ab and CMI responses (21). Furthermore, in H and L pigs of G2 and G3, monocyte superoxide anion production, an indicator of monocyte and macrophage function, was found not to vary by line (12). Uptake and killing of *Salmonella typhimurium* by cultured blood monocytes also did not differ between H and L pigs (22). Therefore, differences between H and L pigs in growth and survival of *M. hyorhinis* in SF may not be related to differences in macrophage function. However, differences may exist between H and L pigs in the regulation of inflammation and *M. hyorhinis*-related resistance mechanisms by anatomical microenvironment. It is suggested that surface-variable lipoproteins of *M. hyorhinis* influence the interaction of these organisms with cells by changing the surface charge of

the organisms (28). Lipoproteins may thus help mycoplasmas to adapt to different conditions during pathogenesis. Whether or not such variation occurs preferentially in high responders is not known. It has recently been reported that IL-4-deficient mice have decreased numbers of *Staphylococcus aureus* organisms in joints and less septic arthritis in association with a T-helper 1 cytokine environment which is conducive to more bactericidal macrophages (13). Equivalent circumstances may prevail in L pigs.

The severity of arthritis is greater in pigs selected for high combined Ab and CMI responses, in which there is a trend toward increased joint-associated IL-6 and IFN-γ mRNAs. Thus, *M. hyorhinis* infection of immune response-selected pigs may provide an opportunity to investigate the immunopathogenesis of infectious arthritis as well as possible cytokine-based interventions. Nevertheless, positive correlations between the cytokines measured here and lesion or CFU scores in L pigs but not H pigs may suggest that the

expression of other unidentified key regulatory cytokines may differ by line.

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