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Alterations in Cytokines and Effects of Dexamethasone Immunosuppression during Subclinical Infections of Invasive *Klebsiella pneumoniae* with Hypermucoviscosity Phenotype in Rhesus (*Macaca mulatta*) and Cynomolgus (*Macaca fascicularis*) Macaques

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Invasive *Klebsiella pneumoniae* with the hypermucoviscosity phenotype (HMV *K. pneumoniae*) is an emerging human pathogen that also has been attributed to fatal multisystemic disease in African green monkeys at our institution. Combining a cluster of subclinically infected macaques identified in March and April 2008 and the animals documented during a subsequent survey of more than 300 colony nonhuman primates yielded a total of 9 rhesus macaques and 6 cynomolgus macaques that were subclinically infected. In an attempt to propagate the responsible HMV *K. pneumoniae* strain, a subset of these animals was immunosuppressed with dexamethasone. None of the treated animals developed clinical disease consistent with the multisystemic disease that affected colony African green monkeys. However, cytokine analysis revealed significant alterations of secreted cytokines in macaques subclinically infected with HMV *K. pneumoniae* when compared with noninfected macaques, thereby calling into question the suitability of animals subclinically infected with HMV *K. pneumoniae* for use in immunologic or infectious disease research.

Abbreviations: HMV, hypermucoviscosity phenotype; *rmpA*, regulator of the mucoid phenotype gene; *magA*, mucoviscosity-associated gene.

Klebsiella pneumoniae is a gram-negative member of the Enterobacteriaceae family that comprises part of the normal fecal and oral flora of many nonhuman primates[19] but also has been implicated in cases of peritonitis, septicemia, pneumonia, and meningitis in both Old and New World primates.[17,20,37] Over the past 20 y, strains of invasive *K. pneumoniae* with a unique hypermucoviscosity phenotype (HMV *K. pneumoniae*) have been reported to cause community-acquired primary liver abscesses, meningitis, and endophthalmitis in humans in Taiwan and other Asian countries,[10, 26-31,33,44,48,51] mostly in people with diabetes mellitus.[7,8,44] In addition, HMV *K. pneumoniae* has caused clinical disease in the United States and other nonAsian countries.[18,30,33] The HMV phenotype is determined based on a positive string test, which is performed by touching a colony with a bacterial loop and gently lifting. If a mucoid 'string' of at least 5 mm forms, the string test is considered positive.[3,14,45,51]

Capsular serotypes K1 and K2 have been reported as the major virulence determinants for human HMV *K. pneumoniae* liver abscesses.[9,15,49,50] The products of the mucoviscosity-associated gene (*magA*), which encodes a structural outer membrane protein of the K1 serotype, and the regulator of the mucoid phenotype gene (*rmpA*) have also been proposed as virulence factors.[16,34,42,52,53]

HMV *K. pneumoniae* has been reported to cause multisystemic abscesses in African green monkeys (*Chlorocebus aethiops*).[45] In late 2005 and early 2006, 7 African green monkeys in the research colony at our institution, the US Army Medical Research Institute for Infectious Diseases, were found to have abscesses in multiple locations; all 7 animals either succumbed or were euthanized because of poor prognosis due to surgically nonresectable abdominal abscesses.[45] The etiology of the final case was determined to be HMV *K. pneumoniae* with the K2 serotype and *rmpA*, and all 6 other cases had similar clinical, microbiologic, and pathologic characteristics. Prior to the current study, we believe these 7 cases were the only documented natural infections attributed specifically to HMV *K. pneumoniae* in nonhuman primates.[45]

As a result of those findings, our institution instituted a policy to report *K. pneumoniae* positive cultures in nonhuman primates during quarantine periods and on routine semiannual examination. Over several months in spring and summer 2008, a group of 19 macaques tested positive on oropharyngeal or rectal culture for HMV *K. pneumoniae*; 15 of those 19 animals were isolated in a single room for 2 to 4 mo to better characterize the infection.[3]

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None of the animals showed clinical signs of disease during the isolation period, and abdominal palpation failed to suggest the presence of abdominal abscesses like those seen in African green monkeys. Testing of isolates suggested that the macaques harbored subclinical infections and that multiple genotypes of HMV *K. pneumoniae* were present.[3]

In July 2008, a cynomolgus macaque from the colony that was experimentally challenged with monkeypox virus survived beyond the normal time-to-death window (12 to 16 d after infection). However, on day 22 after infection (6 to 10 d beyond this window), this macaque died unexpectedly. Histopathologic analysis of tissues from this NHP revealed a concurrent gram-negative bacterial infection, based on Gram stains and immunohistochemistry. Although cultures were not available, PCR analysis of DNA extracted from formalin-fixed, paraffin-embedded tissues revealed the presence of *K. pneumoniae* through the amplification of *rmpA*,[39] which is consistent with the HMV phenotype. This animal was considered to have survived infection with monkeypox based on time to death after infection. Monkeypox is reported to target the mononuclear phagocyte system and associated dendritic cells,[54] and we theorized that the monkeypox infection in this macaque led to suppression of the immune system, which then allowed development of a fatal HMV *K. pneumoniae* septicemia.

The present project sought to explore the pathophysiology of HMV *K. pneumoniae* in macaques. We hypothesized that immunosuppression of subclinically infected macaques would produce lesions similar to those observed in the coinfected macaque. In addition, we hypothesized that subclinically infected macaques would have a different immune profile from that of noninfected primates. We measured and analyzed cytokine levels as an indication of altered immune status because such a state potentially could confound research into immunologic responses and infectious disease.

Materials and Methods

Animals. The animals described in this report were maintained in an AAALAC-accredited facility. All research was conducted as part of a protocol approved by our institutional animal care and use committee and adhered to the *Guide for the Care and Use of Laboratory Animals*.[23]

After yielding cultures positive for HMV *K. pneumoniae*, 15 macaques (weight, 3.6 to 7.7 kg; 8 female, 7 male; 9 rhesus macaques [*Macaca mulatta*] and 6 cynomolgus macaques [*Macaca fascicularis*]) were assigned to a single quarantine room in our facility; 14 of these animals cultured positive for HMV *K. pneumoniae* at least twice, either on oral or rectal samples or both. PCR analysis of positive cultures identified $rmpA^+/magA^-$, $rmpA^-/magA^+$, and $rmpA^-/magA^-$ strains in positive macaques.[3] Each animal was clinically normal on physical examination and seronegative for cercopithecine herpesvirus 1, simian retrovirus type D, SIV, and simian T-lymphotropic leukemia virus. No intestinal parasites were detected on fecal examination, either by direct smear or fecal flotation. All of the animals were housed individually in 4.5-ft^2 or 6.0-ft^2 cages with 4 cages per rack (Allentown Caging Equipment, Allentown, NJ), and environmental conditions were maintained as recommended in the *Guide for the Care and Use of Laboratory Animals*[23] (temperature, 16 to 29 °C; humidity, 30% to 70%; and 12:12-h light:dark cycle). Animals were fed a standard primate diet (8714, Harlan Teklad, Madison, WI) supplemented with fruit and other food treats. Fresh water, provided ad libitum, was chlorinated at the municipal level and filtered (Edstrom Industries, Waterford, WI). Environmental enrichment (Challenge Ball, Kong, and Hercules Dental Device, Bio-Serv, Frenchtown, NJ) was provided, and cages were arranged so that the animals were facing each other across the room.

All infected animals were monitored with physical exams, oral and rectal cultures, blood chemistry, and CBC were taken approximately at monthly intervals from first positive culture to the start of immunosuppressive treatment. Once the immunosuppressive protocol was initiated, physical exam, oral and rectal culture, blood chemistry, and CBC were performed weekly. Serum samples were frozen at –80 °C and saved for proinflammatory cytokine profiling.

Immunosuppression. In September 2008, infected macaques were divided into 2 groups (immunosuppressed and nonimmunosuppressed) and matched, to the extent possible, by species, gender, and genotype of HMV *K. pneumoniae* infection. Care was taken to ensure that the macaques selected for immunosuppression were currently yielding cultures positive for HMV *K. pneumoniae*, because several animals had repeated negative cultures and because we suspected that a few of the macaques had cleared the infection.

The immunosuppressed group consisted of 4 infected rhesus and 3 infected cynomolgus macaques, which received dexamethasone subcutaneously (2 mg/kg daily for 3 wk then 1 mg/kg daily for 4 wk) for a total of 7 wk. Dexamethasone was selected for immunosuppression at a dose similar to one used previously to induce clinical signs of Lyme disease in nonhuman primates.[2,4,36] The nonimmunosuppressed group consisted of 5 infected rhesus and 3 infected cynomolgus macaques. All infected macaques were monitored weekly with physical exam, oral and rectal culture, blood chemistry, and CBC. Blood cultures were performed at the beginning of the study and then daily over a 3-d period at its conclusion 7 wk later.

Proinflammatory cytokine profiling of macaques infected with HMV *K. pneumoniae*. Cytokine testing was performed at 2 time points. Before immunosuppression, serum was collected once from each of 15 infected and 15 noninfected (control) macaques that were matched based on species, sex, and approximate weight. After the start of immunosuppression, repeated serum samples were collected from uninfected controls (3 cynomolgus and 4 rhesus macaques), infected immunosuppressed animals (3 cynomolgus and 4 rhesus macaques), and infected nonimmunosuppressed primates (3 cynomolgus and 5 rhesus macaques).

Serum levels were determined by a sandwich immunoassay method using a commercially available electrochemiluminescence detection kit (Human Proinflammatory 9-Plex Kit, Meso-Scale Discovery, Gaithersburg, MD) according to the manufacturer's specifications. The kit assayed the following cytokines: granulocyte–macrophage colony-stimulating factor, IFNγ, IL10, IL12p70, IL1β, IL2, IL6, IL8, and TNFα. The data were $\log_{10}$-transformed for analysis. After transformation, variables were better fitted to assumptions of normality and homogeneity of variance. Therefore, all comparative analyses are for geometric means.

Before immunosuppression, $\log_{10}$-transformed cytokine data from infected (6 cynomolgus and 9 rhesus macaques) and control (6 cynomolgus and 9 rhesus macaques) animals underwent ANOVA using SAS Version 9.2 (SAS Institute, Cary, NC). Covariate analysis of gender and weight was used to determine whether these variables should be included in the model. After initiation of drug-induced

Table 1. Results from HMV *K. pneumoniae* cultures in macaques

Animal	Culture type	Culture results, by date																
		04/07	04/28	05/09	05/20	05/28	06/17	07/21	08/18	09/02	09/09	09/16	09/23	09/30	10/07	10/21	10/28	11/03
Rh2[a]	Oral	+		—	+	—	—	+	—	—	—	—	—	—	—	—	+	—
	Rectal	—		—	+	+	+	+	—	—	+	+	—	—	—	+	—	+
Rh3[b]	Oral	+		—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	Rectal	+		+	+	—	+	+	+	—	—	—	—	—	—	—	—	—
Rh4[b,c]	Oral	+		—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	Rectal	+		+	+	+	—	+	—	—	—	+	—	—	—	—	—	—
Rh5[a,c]	Oral			+	+	+	—	+	+	+	+	—	—	+	—	+	—	—
	Rectal			+	+	+	+	—	+	+	+	+	+	+	+	+	+	+
Rh6[b]	Oral			—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	Rectal			+	—	+	—	—	—	—	—	—	—	—	—	—	+	—
Rh7[a]	Oral			+	+	—	—		—	—	+	+	—	—	—	—	—	—
	Rectal			+	+	+	+		—	+	+	—	+	—	—	+	+	+
Rh8[a,b]	Oral					+	—	—	—	—	—	—	+	—	—	—	—	—
	Rectal					+	+		+	—	+	—	+	—	+	—	—	—
Rh9[b]	Oral					—	+	—	—	—	—	—	—	—	—	—	—	—
	Rectal					—	—	—	—	—	—	—	—	—	—	—	—	—
Rh10	Oral					—	+	—	—	—	—	+	—	+	+	—	+	—
	Rectal					—	—	—	—	—	—	—	—	—	—	—	—	+
Cy1[b]	Oral		—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	Rectal		+	—	+	—	—	—	—	—	—	—	—	—	—	—	—	—
Cy2[a]	Oral		—	—	—	+	+	+	—	—	+	+	—	+	+	—	+	+
	Rectal		+	+	+	+	+	+	+	+	—	+	—	+	—	+	+	+
Cy3[b]	Oral		+	+	+	+	+	—	—	—	—	—	—	—	—	—	—	—
	Rectal		—	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Cy4[a]	Oral		—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	Rectal		+	—	+	—	+	—	+	+	—	—	—	—	+	+	+	+
Cy5[b]	Oral		—	—	+	—	—	+	—	—	—	—	—	—	—	—	—	—
	Rectal		+	+	+	+	+	—	—	—	—	—	—	—	—	—	—	—
Cy6[a,b]	Oral					—	—	—	—	—	—	—	—	—	—	—	—	—
	Rectal					+	+	+	+	+	+	+	+	+	+	—	—	—

Empty cell, culture not performed; –, culture was negative for HMV *K. pneumoniae*; +, culture was positive for HMV *K. pneumoniae.*
Immunosuppressive study began on 09/16/08.
[a]Immunosuppressed
[b]No evidence of HMV *K. pneumoniae* infection at necropsy, as determined by culture and histopathology
[c]Concurrent *Helicobacter* infection identified at necropsy

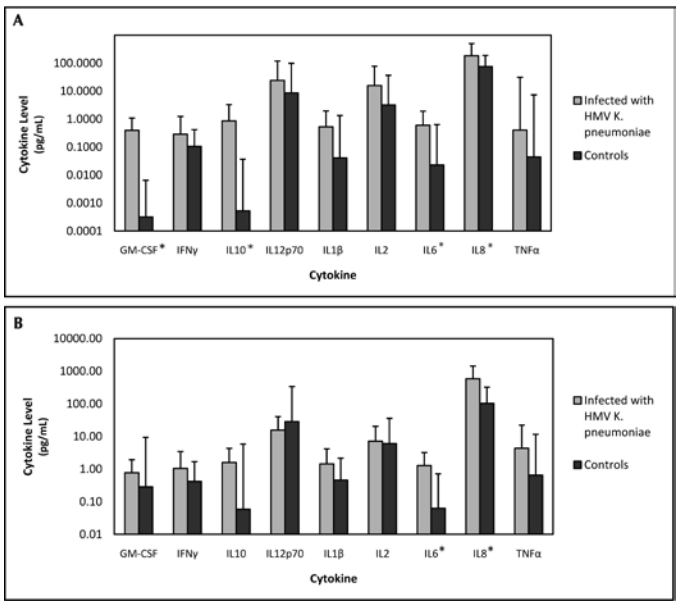

Figure 1. Cytokine levels (geometric mean; bar, SEM) in macaques before immunosuppression. (A) Cynomolgus macaques showed significant (*, $P \leq 0.05$) differences between groups for granulocyte–macrophage colony-stimulating factor (GM-CSF), IL10, IL6, and IL8. (B) Rhesus macaques showed significant (*, $P \leq 0.05$) differences between groups for IL6 and IL8.

immunosuppression, ANOVA with stepdown Sidak adjustment for multiple comparison was performed using SAS Version 9.2 (SAS Institute) on the $\log_{10}$-transformed cytokine data from control (3 cynomolgus and 4 rhesus macaques), infected immunosuppressed (3 cynomolgus and 4 rhesus macaques), and infected nonimmunosuppressed (3 cynomolgus and 5 rhesus macaques) animals.

Pathologic examination. A veterinary pathologist performed complete necropsies all 15 culture-positive HMV *K. pneumoniae*-infected macaques. Cultures were taken at necropsy from the following sites: tonsil, esophagus, stomach, duodenum, jejunum, cecum, ileocecocolic lymph node, and colon. Urine was obtained by cystocentesis at necropsy for urinalysis and culture, and heart blood was taken for culture. Tissues were preserved in neutral-buffered 10% formalin, processed conventionally, embedded in paraffin, cut into 5-µm sections, and stained with hematoxylin and eosin. Special stains, including the standard Lilly Twort staining method and Warthin–Starry silver stain,[11] were used on samples when indicated. The Lilly Twort staining method consists of staining deparaffinized tissue sections in crystal violet for 1 min, rinsing, and then staining with Lugol iodine for 1 min. The tissue sections were counterstained with neutral red–fast green and coverslipped. The Warthin–Starry silver stain consists of rehydrating deparaffinized tissue sections with distilled water and placing sections in a 1% silver nitrate solution and heated in a 60 °C waterbath for 30 min. The slides then are flooded with developer solution (2% silver nitrate, gelatin, and hydroquinone solution) for 15 min. Slides are placed in 56 °C distilled water, dehydrated with alcohol and xylenes, and coverslipped.

Results

Animals. According to repeated oropharyngeal or rectal cultures (or both) positive for HMV *K. pneumoniae*, 6 of the 15 macaques remained subclinically infected for as long as 7 mo. The remaining 9 macaques, including 2 immunosuppressed animals, appeared to clear the infection by the end of the study (Table 1). Physical examinations were unremarkable, with the exception of 1 animal that had periodic episodes of diarrhea. The diarrhea was self-limiting and lasted for no more than 2 to 3 d at a time. In addition, 2 of the immunosuppressed cynomolgus macaques began to have some peripheral edema approximately 1 wk before euthanasia; one had mild peritesticular edema, and the other had moderate peritesticular and mild lower limb edema.

Immunosuppression. Significant ($P < 0.05$) differences were identified between infected immunosuppressed and infected nonimmunosuppressed macaques in several hematology and blood chemistry measurements. In the immunosuppressed group, lactate dehydrogenase, potassium, AST, magnesium, and percentage neutrophils were increased but total protein, albumin, creatinine, percentage eosinophils, percentage basophils, percentage lymphocytes, absolute eosinophils, and absolute lymphocytes were decreased when compared with the nonimmunosuppressed group (data not shown).

Cytokine analysis. The results of the cytokine analysis prior to immunosuppression are provided in Figure 1. The animals in these experiments were selected because of naturally occurring infection; therefore, no randomization based on weight or gender could be made before sample collection for cytokine analysis. Every effort was made to match weight and sex in the uninfected control animals. Statistical analysis was conducted to include the variables of weight and gender, which could not have been accounted for in the study design. Cynomolgus macaques showed significant ($P < 0.05$) interactions between group and gender or weight for several of the cytokine factors (granulocyte–macrophage colony-stimulating factor, IL10, and IL2), and these variables were included in the comparative group analysis for these cytokine factors. Statistically significant ($P < 0.05$) differences between groups were detected for granulocyte–macrophage colony-stimulating factor, IL10, IL6, and IL8. Differences in all other cytokine factors were statistically insignificant. Rhesus macaques showed no significant interactions between group and gender or weight for any of the cytokine factors, therefore these variables were excluded from the comparative group analysis. However, rhesus macaques did display statistically significant ($P < 0.05$) differences between groups for IL6 and IL8 (Figure 1). No other significant differences in cytokine factors were noted.

After immunosuppression, we examined the sera from the uninfected, infected, and infected–immunosuppressed animals for alterations in cytokine secretion. Repeated-measures ANOVA of cytokine measurements between treatment groups for each monkey species were calculated over time, with stepdown Sidak adjustment for multiple comparisons. All cytokine values were $\log_{10}$ transformed for analysis. After transformation, variables were better fitted to assumptions of normality and homogeneity of variance required for parametric analysis. Pairwise comparisons were made for geometric means of cytokine factors over time for the following cynomolgus macaque groups: uninfected controls ($n = 3$), infected animals ($n = 3$), and infected–immunosuppressed animals ($n = 3$). The pairwise comparison for geometric means of cytokine factors over time for rhesus macaque groups included uninfected controls ($n = 4$), infected animals ($n = 5$), and infected–immunosuppressed animals ($n = 4$). Table 2 provides the results of this analysis, giving the P values for the group effect over time, for each pairwise com-

Table 2. *P* values of pairwise comparison of cytokine factors in macaque sera samples after immunosuppression

	Cynomolgus macaques: uninfected controls compared with		Rhesus macaques: uninfected controls compared with	
Cytokine	All infected macaques	Immunosuppressed infected macaques	All infected macaques	Immunosuppressed infected macaques
GM-CSF	0.8765	0.8765	0.9203	0.9389
IFNγ	0.8014	0.9168	0.3368	0.259
IL10	**0.0012**	**0.0429**	0.4836	0.4836
IL12p70	0.7403	0.5837	0.9964	0.9964
IL1β	0.999	0.999	0.6248	0.6239
IL2	0.7441	0.7441	0.4396	0.4396
IL6	0.4989	0.7858	0.2674	0.3052
IL8	0.4728	**0.0001**	**0.0001**	**0.0002**
TNFα	0.3312	0.3312	0.9984	0.9984

GM-CSF, granulocyte–macrophage colony-stimulating factor
Bold face indicates significant ($P \leq 0.05$) differences between groups.

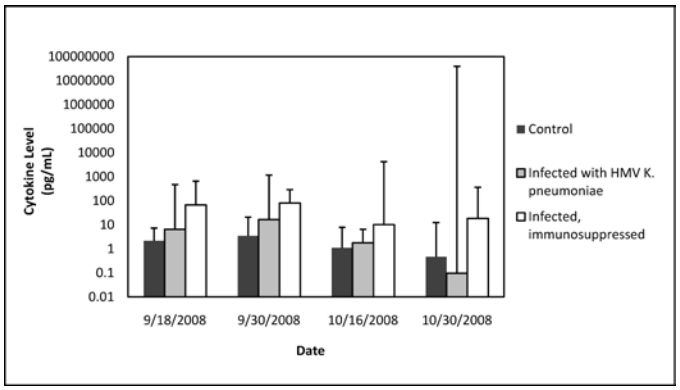

Figure 2. IL8 levels (geometric mean; bar, SEM) in cynomolgus macaques after immunosuppression. Levels differed significantly ($P < 0.05$) between groups.

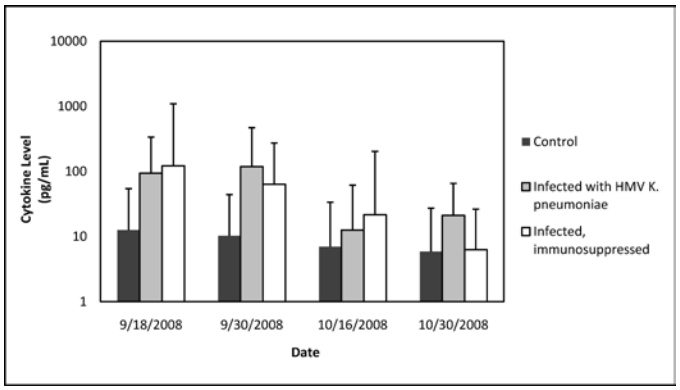

Figure 3. IL8 levels (geometric mean; bar, SEM) in rhesus macaques after immunosuppression. Levels differed significantly ($P < 0.05$) between groups.

parison of the specified cytokines. Cynomolgus macaques showed statistically significant ($P < 0.05$) differences between groups over time for IL10 (data not shown) and IL8 (Figure 2). Rhesus macaques demonstrated statistically significant ($P < 0.05$) differences between groups over time for IL8 (Figure 3).

Pathologic analysis. In the 7 immunosuppressed macaques, the primary gross pathologic change was the presence of multifocal white to chalky areas in abdominal fat (3 of 7 animals) and periteticular edema (2 of 7). Variably present gross changes included splenic size reduction, increased abdominal and pericardial fat, and decreased lymph node size. Among the 7 nonimmunosuppressed macaques, one animal had mild axillary and inguinal lymph node enlargement, and another had a small area of hemorrhage on the caudal rectum at the level of the uterus. No other gross pathologic lesions were noted in any of the nonimmunosuppressed macaques. For all infected macaques, no abnormal findings were detected on urinalysis of urine obtained by cystocentesis at necropsy, and cultures of urine and heart blood were negative.

The most significant histologic changes in the 7 immunosuppressed macaques were lymphoid atrophy in multiple lymph nodes and spleen (5 animals; Figure 4) and atrophy of the zona fasciculata of the adrenal cortex (5 animals; Figure 5). Other histologic changes included mediastinal and mesenteric fat necrosis (3 animals), epicardial and subcutaneous fat necrosis (1 animal), mild interstitial nephritis (1 animal), glycogen-type vacuolar change in liver (1 animal), and typhlocolitis with myriad gram-negative bacilli and *H. pylori* gastritis (1 animal; Figure 6). Findings from microscopic analysis of tissues from the 7 nonimmunosuppressed macaques included mild to moderate lymphoid hyperplasia of multiple lymph nodes, spleen, and gut-associated lymphoid tissue (6 animals) and lymphoplasmacytic gastroenteritis (1 animal).

Helicobacter was detected in the gastrointestinal tract of 2 infected macaques (one immunosuppressed and one nonimmunosuppressed). Both animals had large numbers of organisms evident during histopathologic analysis of silver-stained slides. Diarrhea was noted transiently in the immunosuppressed *Helicobacter*-infected macaque, and histology revealed lymphoplasmacytic gastroenteritis in the nonimmunosuppressed macaque.

Discussion

In light of invasive disease in colony African green monkeys that was attributed to infection with HMV *K. pneumoniae*, the

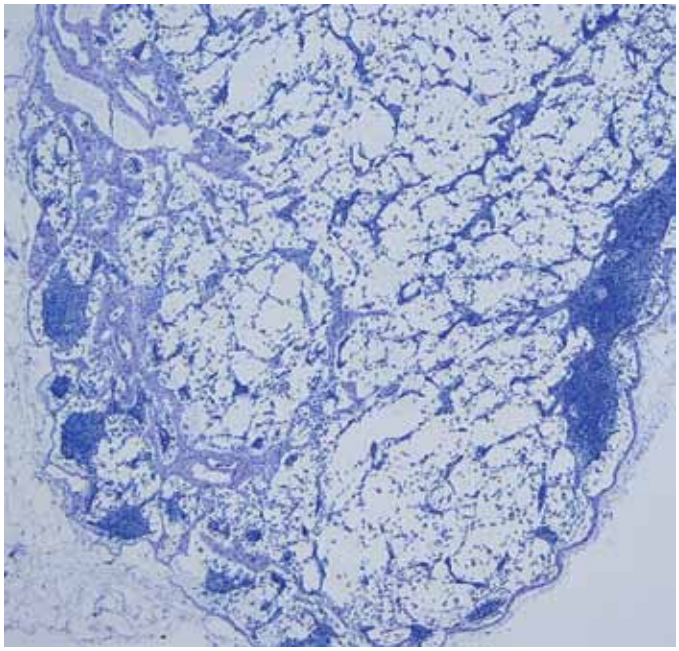

Figure 4. Mesenteric lymph node from a glucocorticoid -treated macaque. Note the diffuse paucity of lymphoid tissue (lymphoid atrophy). Hematoxylin and eosin stain; magnification, ×4.

finding of infected macaques raised concerns about the effect on research and possibility of clinical manifestation of disease. In our study, a total of 15 macaques were found to be infected with HMV *K. pneumoniae*, and they were isolated and clinically monitored for as long as 7 mo.

In an effort to determine some of the potential effects of this subclinical infection on infectious disease research, we performed assays to determine whether cytokines varied between subclinically infected macaques and uninfected controls. Levels of granulocyte–macrophage colony-stimulating factor, IL10, IL6, and IL8 were increased ($P < 0.05$) in infected cynomolgus macaques, and IL6 and IL8 concentrations were increased ($P < 0.05$) in infected rhesus macaques. In addition, IL10 and IL8 levels were increased ($P < 0.05$) in both immunosuppressed and nonimmusuppressed cynomolgus macaques, and IL8 was higher ($P < 0.05$) in immunosuppressed and nonimmunosuppressed rhesus macaques compared with uninfected species-matched macaques over time.

The cytokine granulocyte–macrophage colony-stimulating factor is secreted by activated T cells, mast cells, endothelial cells, basophils, and macrophages. It stimulates the growth of hematopoietic progenitors, stimulates the phagocytic and intracellular killing mechanisms of neutrophils, increases the phagocytic capacity of monocytes and macrophages,[13] inhibits differentiation of $CD34^+$ progenitor cells into type 2 dendritic cells, and inhibits terminal differentiation of mast cells.[32] Granulocyte–macrophage colony-stimulating factor has a physiologic role in allergic inflammation, helps to modulate pulmonary alveolar macrophage activity, and has been implicated in transduction of signals promoting survival and proliferation of transformed neoplastic cells.[32]

IL10 is an antiinflammatory interleukin that can downregulate cellular immunity by suppressing the production of various proinflammatory mediators.[46] It is produced primarily by macrophages and to a lesser extent by T cells, monocytes, dendritic cells, B cells, eosinophils, mast cells, and keratinocytes.[46] IL10 plays a pivotal role in immunoregulation during viral, bacterial, fungal, protozoal, and parasitic infections by tempering Th1 and $CD8^+$ T cell responses and preventing the overproduction of IFNγ and TNFα that frequently results in severe complications from these conditions.[12] In some cases, dysregulation of IL10 can result in pathogens escaping immunologic control and causing severe disease.[12]

IL6 was originally considered to be a B-cell differentiation factor. Today, however, it is known to regulate many functions in immune response, hematopoiesis, acute phase response, and inflammation.[24] IL6 can act in either an inflammatory or an antiinflammatory capacity, depending on its physiologic environment. This cytokine is secreted by macrophages during acute inflammation and by T cells during chronic inflammation; under stress conditions, blood serum levels of IL6 can rapidly rise.[35] Toll-like receptors are important sensor units of the innate immune system that can recognize specific antigen patterns of broad classes of microbes, and the activation of these receptors induces an intracellular signaling cascade that leads to increased production of IL6.[35] This process can, in turn, induce the production of other inflammatory cytokines, such as IL8. Interestingly, a study in mice found that mast-cell–derived IL6 has a protective role against *K. pneumoniae* infection and sepsis and improves intracellular neutrophil killing of *K. pneumoniae*.[43]

Despite its proinflammatory properties, IL6 probably takes on a larger role as an antiinflammatory cytokine, given its ability to induce hepatic production of acute-phase proteins. These proteins have a protective effect by limiting inflammation through antiprotease and scavenger activities.[6] In addition, IL6 helps to orchestrate the transition from innate to acquired immune responses, and it is involved in the cascade that determines whether T cells become suppressors or activators of the adaptive immune system.[25,35] In recent years, dysregulation of IL6 has been reported to be an important factor in diseases of chronic inflammation, such as obesity and insulin resistance, autoimmune diseases such as inflammatory bowel disease and rheumatoid arthritis, and inflammation-associated cancers.[35]

IL8 is a powerful cytokine that is primarily responsible for activating neutrophils upon exposure to inflammatory stimuli. It is produced by macrophages and many other cell types, including epithelial cells, endothelial cells, fibroblasts, keratinocytes, synovial cells, chondrocytes, hepatocytes, gastric cancer cells, and even neutrophils.[1,21,22] As a product of many cell types, IL8 can arise in any tissue when levels of other inducing cytokines are increased; in addition, it is resistant to inactivation and has a slow clearance.[1] Some of these inducing cytokines are stimulated by activation of pattern recognition receptors on the cell surface, mainly Toll-like receptors.[35] These receptors recognize pathogen-associated molecular patterns, such as LPS in gram-negative bacteria, and induce an intracellular signaling cascade that leads to increased production of cytokines, including IL8.[35] IL8 has a number of effects on neutrophils, upregulating responses required for migration and phagocytosis. It induces shape change, releases lysosomal enzymes, induces respiratory burst, improves adhesion to endothelial cells, and generates hydrogen peroxide and superoxide.[22] Although the primary function of IL8 is to recruit neutrophils to phagocytose antigens, it also induces chemotaxis in other cells, including T cells and basophils.[22]

Although the cytokine alterations in macaques infected with HMV *K. pneumoniae* seem intriguing, assigning clinical signifi-

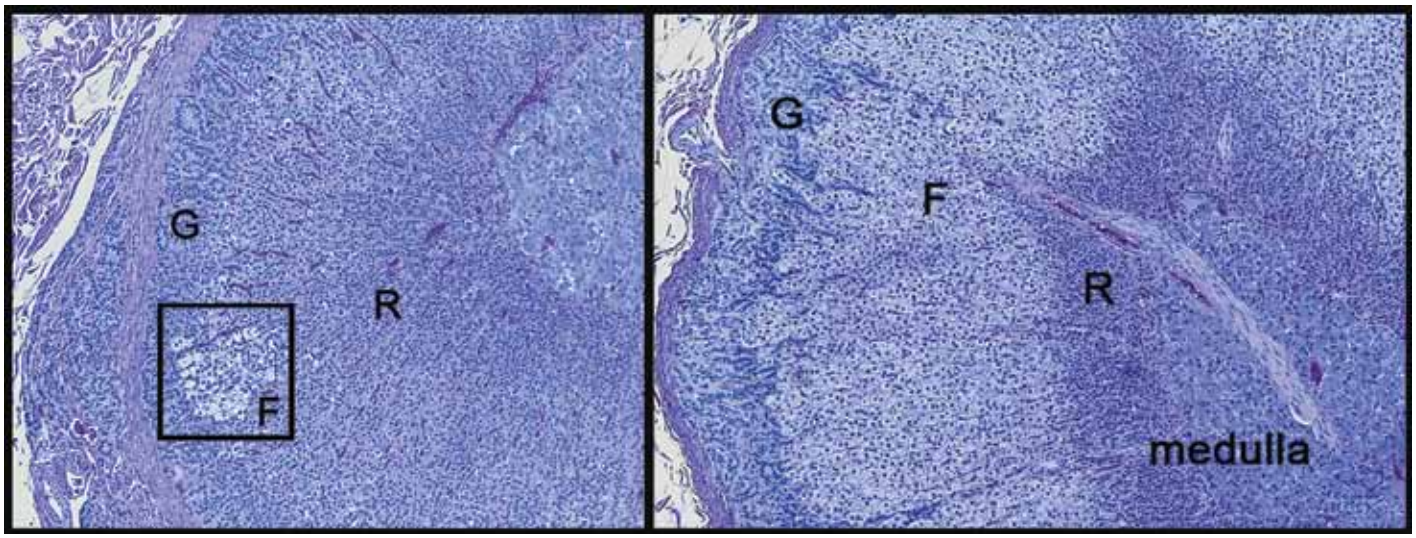

Figure 5. Adrenal gland from a glucocorticoid-treated macaque (left) shows diffuse atrophy of zona fascicularis, with only a small portion (boxed area) remaining. Adrenal gland from a nonimmunosuppressed macaque (right). F, zona fascicularis; G, zona glomerulosa; R, zona reticularis. Hematoxylin and eosin stain; magnification, ×20.

Figure 6. Stomach from a nonimmunosuppressed macaque. Note the helical agyrophilic bacteria (arrows) lining the gastric epithelium. Warthin–Starry silver stain; magnification, ×40.

cance to these effects is difficult due to the extremely complex interrelationships between cytokine production and immunologic function. However, many infectious disease studies that focus on specific immunologic markers and cytokine influences can be disrupted by subclinical infections with organisms such as HMV *K. pneumoniae*, even if the infection does not cause clinical signs of disease. Examples of other potentially confounding infections in macaques include simian parvoviruses, the polyomavirus simian virus 40, and simian lymphocryptoviruses. These viruses cause only mild or inapparent clinical disease in immunocompetent animals but may result in immunologic dysfunction that complicates many types of immunologic research.[5,40,41]

The unexpected finding of a macaque that succumbed to a *K. pneumoniae* septicemia after surviving an experimentally induced monkeypox infection underscored the need to further characterize the disease-causing potential of HMV *K. pneumoniae* in macaques. It was previously determined that multiple genotypes of HMV *K. pneumoniae* were responsible for the subclinical infections in infected nonhuman primates.[3] With the exception of mild, transient diarrhea that lasted for no more than 2 to 3 d at a time and that likely was due to concurrent *Helicobacter* infection in 1 rhesus macaque, no clinical signs of disease were apparent in any of the macaques determined to be infected with HMV *K. pneumoniae*.

The immunosuppressive effects of the exogenous glucocorticoid (dexamethasone) therapy used in this study have long been recognized; however, the mechanism of action of glucocorticoids is complex and incompletely characterized. This class of drugs directly or indirectly regulates the expression of many genes, and its inhibition of cytokine production is generally accepted as one of the most important factors contributing to glucocorticoid-induced immunosuppression.[38] Glucocorticoids are reported to inhibit the gene transcription of several cytokines, including IL8, by repressing the activity of an essential transcription factor, NFκB.[22,47] Furthermore, despite a dose of dexamethasone higher than one reported previously to immunosuppress macaques,[36] all immunosuppressed macaques in our study still showed higher IL8 levels than those of uninfected controls. This finding may help to explain our inability to elicit clinical signs of disease associated with HMV *K. pneumoniae* with our immunosuppressive protocol, or it may point to some poorly characterized mechanism of action of glucocorticoids in which IL8 is induced via an indirect pathway which is outside the influence of glucocorticoids. It is noteworthy that 2 macaques (Rh8 and Cy6 in Table 1) actually cleared the infection while under the effects of immunosuppressive therapy.

In future studies, it would be advantageous to incorporate fluorescence-activated cell sorting in an effort to determine which populations of cells were altered during dexamethasone administration. We attribute blood chemistry, hematology, and gross and histologic changes directly to the immunosuppressive effects of glucocorticoids, including lymphoid atrophy (Figure 1) and marked atrophy of the zona fascicularis in the adrenal glands (Figure 2). In addition, immunosuppressed macaques showed glucocorticoid (or 'stress') leukograms.

This study is the first to examine the implications of subclinical infection with HMV *K. pneumoniae* in macaques. The subclinical infection appeared to have minimal clinical significance in macaques. Despite some suggestion of clinical disease in a macaque that was coinfected with monkeypox, we found no evidence of clinical disease in infected, glucocorticoid-immunosuppressed macaques. However, infected macaques showed significant alterations in cytokines as compared with uninfected macaques, suggesting poor suitability of animals infected with HMV *K. pneumoniae* for use in immunologic or infectious disease research. Further research efforts should focus on characterization of this infection in African green monkeys, which are particularly susceptible.

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