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Disseminated disease severity as a measure of virulence of *Mycobacterium tuberculosis* in the guinea pig model

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Abstract

Virulence is the measure of pathogenicity of a microorganism as determined by its ability to invade host tissues and to produce severe disease. In the low-dose aerosol guinea pig model the virulence of multiple strains of *Mycobacterium tuberculosis* was determined by measuring time of survival, bacterial loads in target organs, and the severity of pulmonary and extra-pulmonary lesions. Erdman K01, CSU93/CDC1551 and HN878 had shorter survival times compared to the common laboratory strain H37Rv. After thirty days of the infection bacilli had disseminated from the lungs resulting in microscopically visible lesions in peribronchial lymph nodes, peripancreatic lymph nodes, spleen, liver, pancreas, adrenal and heart. The extent of the lesion necrosis paralleled virulence when survival times were used as a measure as Erdman K01 and the two clinical isolates caused more necrosis and resulted in sooner death in infected animals than the H37Rv. The extent of extra-pulmonary lesion necrosis was a better predictor of virulence than the number of viable bacilli in the tissue. Overall, this study emphasizes the point that extra-pulmonary disease is a prominent feature of the guinea pig model and dissemination to organs not normally assayed such as the heart and adrenal glands should be taken into account in the assessment of the disease process.

Keywords

Mycobacterium tuberculosis; clinical isolates; macrophages; pathology; pathology; guinea pigs; granuloma; inflammation

Introduction

Tuberculosis is one of a small group of diseases that together cause 90% of infectious disease related deaths worldwide. *M. tuberculosis*, the causative agent of tuberculosis, infects approximately 8 million new individuals per year and the disease results in a death in every ten seconds^{1,2}. Despite the WHO declaring tuberculosis as a global health emergency nearly fifteen years ago, no significant progress in eradication of the disease has been made. Multiple factors such as poor socioeconomic conditions, limited access to diagnosis and treatment, lack of treatment compliance and the development of multidrug-resistant *M. tuberculosis* strains have contributed to this lack of progress. In addition, different clinical isolates of *M.*

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tuberculosis may undergo genetic changes and result in differences in pathogenicity and variations in the ability to cause disease in humans ³.

Virulence is defined as the severity of disease symptoms resulting in death of the infected host. Virulence of *M. tuberculosis* strains is generally determined by the differences in survival in experimentally infected mice or guinea pigs ⁴. Since the survival studies are lengthy and expensive, the number of cultivatable bacilli in lungs is alternatively used in short term experiments as a correlate of virulence ^{5, 6}. Weight loss and clinical signs are considered the most common correlates of disease progression ⁷. Antigen-specific delayed-type hypersensitivity (DTH) ⁸ and antigen-induced production of IFN- γ by T cells ⁹ have also been used as the determinants of protective immunity with IFN- γ production generally being considered a better correlate of resistance in mice ¹⁰. As tuberculosis in guinea pigs, like other animal models, is a systemic disease with morbidity and mortality associated with pulmonary and extra-pulmonary pathology, we compared the virulence of clinical and laboratory isolates of *M. tuberculosis* based on lesion burden of the different body organs, the survival times and the number of culturable bacilli.

M. tuberculosis CSU93/CDC1551 is a clinical isolate that exhibited high levels of infectivity and virulence during an outbreak of tuberculosis from 1994 to 1996 in rural Tennessee and Kentucky ¹¹. It was characterized by an unusually high rate of tuberculin skin test conversions as well as cases of active disease. Many of these patients developed active disease following very brief exposures to the index case ¹¹. CSU93/CDC1551 was originally shown to grow to very high numbers in mice lungs (>107 per lung) but later observations indicated that properly passaged laboratory strains can reach a similar level of growth ^{12, 13}. Survival studies carried out in different laboratories using mice did not show any increase in virulence of CSU93/CDC1551 compared to H37Rv ^{12, 14, 15}.

M. tuberculosis HN878, a member of the W/Beijing family, caused 60 cases of TB during three outbreaks in Texas between 1995 and 1998 ¹⁶. HN878 was demonstrated to be hyper-virulent in mice based on comparative survival studies ¹⁷. The hyper-virulence of HN878 was suggested to be due to the failure of this strain to stimulate CD4 T cell mediated Th1 type immunity associated with increased induction of Type-I interferon ^{12, 17}. The same researchers later showed that a cell-wall phenolic glycolipid antigen expressed by HN878 was capable of inducing the Th2 type immunity and its absence resulted in abrogation of the strain's hyper-virulence ^{3, 18}. A recent study from our laboratory has confirmed the hyper-virulence of HN878, but instead showed that this organism induced a very substantial Th1 response and this response then declined in concert with the emergence of a CD4+ CD25+ FoxP3+ CD223 + IL10+ regulatory T cell population ¹⁹.

In the present study we compared the above strains in the guinea pig model, taking into account the extensive extra-pulmonary dissemination that may cause premature death. Our data shows that the rate of dissemination and the extent of extra-pulmonary pathology explain in part the differences in the virulence of *M. tuberculosis* isolates in this important animal model.

Materials and methods

Experimental infections

Female out-bred Hartley guinea pigs (approximately 500g in weight) were purchased from the Charles River Laboratories (North Wilmington, MA, USA) and held under barrier conditions in a Bio-safety Level III animal laboratory. All experimental protocols were approved by the Animal Care and Use Committee of Colorado State University and comply with NIH guidelines.

M. tuberculosis H37Rv was originally obtained from the Trudeau Institute collection, Saranac Lake NY; HN878 was kindly provided by Dr. Barry Kreiswirth; CDC1551/CSU93 was provided by Dr. T. Shinnick, CDC, Atlanta; and Erdman-K01 was obtained from Mycos Research, Loveland CO with permission from the Aeras Global Tuberculosis Vaccine Foundation. Cultures were aliquoted into 1 ml tubes and frozen at -70°C until used. Thawed aliquots were diluted in milli-Q sterile water to the desired inoculum concentrations. A Madison chamber aerosol generation device was used to expose the animals to *M. tuberculosis*. This device was calibrated to deliver approximately 20 bacilli into the lungs.

Bacterial counts in the lungs, spleen and peribronchial lymphnodes (n=5) on day 30 were determined by plating serial dilutions of organ homogenates on nutrient 7H11 agar and counting colony-forming units after 3 weeks incubation at 37°C . In survival studies animals showing substantial weight loss with no evidence of weight rebound were euthanized. The results shown in the survival studies are based upon 6 guinea pigs per group.

Tissue fixation and processing

At the time of euthanasia either on day 30 post-infection or at the end of survival study a necropsy was performed. Organs which show extra pulmonary tuberculosis infection in human were collected from these animals and fixed in 10% neutral buffered formalin (NBF) for 3 days. The tissues were then trimmed and processed for paraffin embedding and 4μ thick sections were made, stained with hematoxylin and eosin and assessed histopathologically. The photomicrographs presented here are representatives of their respective groups based on mean lesion scores and are taken using an Olympus BX41 microscope with Olympus DP70 camera and software.

Lesion analysis

To evaluate the concurrent progression tuberculosis lesions in lungs, lymph nodes, spleen, liver, pancreas, adrenal and heart different histological grading systems were developed. The method was a modification of previously described methods for grading granulomatous lesions based on inflammatory cell numbers and their infiltrative distribution pattern ²⁰.

The guinea pig lungs were scored based on the following six criteria. (i) percent of lung affected were ranked at low magnification by an estimation of the percent of the lung affected as follows: 0-no lesions in lung, 1-up to 25% of lung involved, 2-up to 50 % of lung involved, 3-up to 75% of lung involved, 4-above 75% of lung involved. (ii) primary lesions: 0-no primary lesions present, 1-a single primary lesion, 2-two or more primary lesions, multi-focal, 3-two or more primary lesions, multifocal to coalescing, 4-multiple primary lesions, coalescing and extensive. (iii) secondary lesions: 0-no secondary lesions present, 1-up to 25% of lung involved, 2-up to 50% of lung involved, 3-up to 75% of lung involved, 4-above 75% of lung involved. Necrosis (iv), mineralization (v) and fibrosis (vi) are scored based on severity as follows: 0-none, 1-minimal, 2-mild, 3-moderate, 4-marked and 5-severe. All individual scores were added for the final total score for each organ. Maximum possible total score for lungs is 27.

The peribronchial and peripancreatic lymph nodes, liver and spleen were scored based on four categories; (i) percentage involvement (same scale as lungs), (ii) degree of necrosis, (iii) fibrosis and (iv) mineralization (0–5 scale). The maximum total score for each of these organs is 19. The lesions in other organs such as heart, kidney and adrenal were scored based on severity only with the 0–5 degree scale as shown above.

Statistical analysis

Data is presented as mean values (n=6). The parametric log rank test was used to assess statistical significance between survival curves. Statistical analysis to evaluate whether the

total lesion scores and necrosis scores showed statistically significant difference was done using oneway ANOVA (GraphPad Prism software).

Results

Evaluation of virulence of *Mycobacterium tuberculosis* strains

Based on survival as shown in Figure 1A and 1B, among the four strains tested, Erdman K01 was the most virulent strain in guinea pigs followed by CSU93/ CDC1551. The survival rate of both Erdman K01 and CSU93/CDC1551 demonstrated statistically significant differences from that of the H37Rv infected animals ($p<0.05$). The other clinical isolate, HN878, was the third most virulent strain behind Erdman K01 and CSU93/CDC1551.

The number of viable bacilli in lungs, spleen and peribronchial lymph nodes on day 30 of the infection is shown in Figures 1C, D and Figure E. In both the lungs and peribronchial lymph nodes, HN878 showed marginally higher number of bacilli compared to the other three strains. In terms of bacterial dissemination and growth in the spleen, the Erdman K01 strain grew slightly higher than the other strains that grew to similar levels.

Lung histopathology

The differences in the relative size and lesion progression on day 30 in the lungs of guinea pigs infected with H37Rv (Figure 2A), Erdman K01 (Figure 2B), CSU93 (Figure 2C) and HN878 (Figure 2D) on day 30 are shown. In addition, the mean total lesion scores for each group were calculated as described in the materials and methods section (Table 1).

On day 30 of the infection with all four bacterial strains, the lung lesions (Figure 2 A–D) consisted of well delineated foci of granulomatous inflammation characterized by sheets of epithelioid macrophages and occasional multinucleated giant cells mixed with fewer numbers of lymphocytes, plasma cells and occasional neutrophils and eosinophils with moderate amount of necrosis (data not shown). As the infection progressed and animals started to die, lesions contained equal numbers of macrophages and lymphocytes and extensive necrosis, fibrosis and mineralization of central necrotic cores (data not shown). Erdman K01, CSU93 and HN878 caused more lymphatic-associated lesions around the airway (data not shown) and more necrosis (Figure 2 A–D; arrows) on day 30 resulting in higher lesion scores compared to H37Rv. At death, necrosis was comparatively more extensive in lungs of guinea pigs infected with Erdman K01, CSU93 and HN878 compared to H37Rv and the mineralization of the necrotic core was most predominant in the Erdman K01 and HN878 infected animals compared to the other two strains (Table 2).

Peribronchial lymph nodes histopathology

The peribronchial lymph nodes of the infected guinea pigs showed remarkable lesion development and gross enlargement regardless of which *M. tuberculosis* strain was used. A mixed inflammatory response involving lymphocytes, macrophages and granulocytes (neutrophils and eosinophils) was present in all four groups resulting in granulomatous lymphadenitis (Figure 2 E–H). On day 30, Erdman K01, CSU93 and HN878 caused moderately more necrosis in the peribronchial lymph nodes than H37Rv. At the time of death, H37Rv infected lymph nodes showed less necrosis but extensive fibrosis compared to Erdman K01, CSU93 and HN878 (data not shown). In the peribronchial lymph nodes on day 30 of the infection, the necrosis scores (Table 2) paralleled the survival times (Figure 1). At the time of death, the peribronchial lymph nodes in the HN878 infected animals had the most severe necrosis followed by Erdman K01 and CSU93 (Table 2).

Peripancreatic lymph nodes histopathology

The peripancreatic lymph nodes (Figure 3 A-D) from all four groups showed lesion progression similar to that of the peribronchial lymph nodes and the degree of granulomatous and necrotizing lymphadenitis was comparable. On day 30, the animals infected with Erdman K01, CSU93 and HN878 showed moderately more necrosis in these lymph nodes than in H37Rv infected animals. At the time of death, H37Rv infected peripancreatic lymph nodes had less necrosis but extensive fibrosis compared to those infected with Erdman K01, CSU93 and HN878 similar to peribronchial lymph nodes (data not shown). In contrast to the peribronchial lymph nodes, the peripancreatic lymph nodes from guinea pigs infected with Erdman K01, CSU93 and HN878 however showed marked mineralization (Table 2). Both at day 30 and at the time of death, the necrosis scores of the peripancreatic lymph nodes did not parallel the survival times. However, the necrosis scores of the peripancreatic lymph nodes from the groups infected with Erdman K01, CSU93 and HN878 were still consistently higher compared to those from the group infected with H37Rv (Table 2).

Spleen histopathology

Figure 3 (E-H) shows the extent of spleen lesions in all four groups on day 30. The lesion scores in Table 1 reflected more spleen lesion development in Erdman K01 and HN878 infected guinea pigs on day 30 compared to H37Rv and CSU93 infected guinea pigs. At the time of death, the spleens from the three groups other than H37Rv had slightly more mineralization (data not shown). The degree of necrosis was almost equal at death in all four groups (Table 2). The necrosis scores of the spleens, however, paralleled the survival times on day 30 of the infection, as was the case for the peribronchial lymph nodes.

Liver histopathology

On day 30, the livers of all four groups showed moderate involvement with few foci of granulomatous inflammation scattered randomly throughout the parenchyma. At this time, there were no differences in liver pathology among the four groups (data not shown). But at the time of death, livers from the guinea pigs infected with Erdman K01, CSU93 and HN878 showed more extensive lesion development and necrosis compared to H37Rv (Figure 4 A-D and Table 2). In the liver, the necrosis scores were similar among the different strains at day 30. However, the scores seen in the H37Rv infected animals were moderately lower.

Differences in other organs and notable lesions

At the time of death, granulomatous pancreatitis was severe in CSU93 infected guinea pigs, moderate in both Erdman K01 and HN878 infected guinea pigs and mild in H37Rv infected guinea pigs (Figure 4 E-H).

Heart (Figure 5 A-D) and adrenal glands (Figure 5 E-H) also had mixed inflammation at the time of death. Granulomatous myocarditis was noticeable even on day 30 with Erdman K01 infected group showing marked inflammation (Figure 5B and Table 1). CSU93 and HN878 caused moderate and H37Rv caused very minimal inflammation (Figure 5 A-D and Table 1). Granulomatous adrenalitis was moderate both in Erdman K01 and HN878 infected groups; mild in the H37Rv infected group and very minimal in the CSU93 infected group (Figure 5 E-H and Table 1).

Focal granulomatous lesions in the colon and the uterus were seen occasionally (data not shown). These were isolated findings. The intestinal inflammation was primarily localized to the gut-associated lymphoid tissue and was composed of predominantly macrophages and lymphocytes with occasional granulocytes in granulomatous lesions.

Statistical analysis to evaluate whether the total lesion scores and necrosis scores showed statistically significant difference revealed that among mean total lesion scores, PBLN scores on day 30, liver scores on day 30 and the pancreas scores at the time of death were the only ones that did not show statistically significant difference. Among necrosis scores, spleen scores at the time of death, liver scores on day 30 and at the time of death were the only ones that did not show statistically significant difference. All other scores showed statistically significant difference among different groups ($p < 0.05$).

The acid-fast staining revealed the tuberculous bacilli to be present in highest concentration in the necrotic core of the lesions followed by the macrophages immediately surrounding the necrotic core (data not shown).

Discussion

The results of the survival study indicated that the Erdman K01 strain was the most virulent strain in the guinea pigs and H37Rv was the least virulent strain of the four tested. The order of virulence was Erdman K01, CSU93/CDC1551, HN878 and finally H37Rv. Despite the slightly higher growth of bacilli in the lungs and the peribronchial lymph nodes in the HN878 infected animals compared to the other strains, the HN878 strain was in fact less virulent than Erdman K01 and CSU93 in the survival studies. Interestingly, the number of culturable bacilli (CFU) in the lungs, spleen and peribronchial lymph nodes did not correlate with the virulence of the strains as determined by survival. This is an important observation since comparison of CFUs in the lungs is often used as a major readout in challenge experiments and as a measure of virulence and vaccine efficacy¹⁰. Our data supports an earlier study in mice that showed that growth rate by itself was an unreliable indicator of mycobacterial virulence¹⁵.

Our results differ from the studies in mice in which HN878 was demonstrated to be more virulent than CSU93 or H37Rv (6⁷). These previous studies demonstrated that the CSU93/CDC1551 infected mice survived significantly longer than HN878 and even H37Rv. Our earlier study in mice also confirmed the increased virulence of HN878¹⁹. In that study even though HN878 grew faster reaching a high bacterial load of 10^7 as early as day 15 of the infection, yet death was delayed. In that study HN878 induced a population of CD4⁺ CD25⁺ regulatory T cells that were shown to suppress the protective effects of Th1 dominant immunity. These findings demonstrate that the virulence as reflected by survival is not predictable by tissue bacterial load alone, which was supported in this study. The guinea pigs showed both HN878 and CSU93/CDC1551 to be more virulent than the laboratory strains and CSU93/CDC1551 to be more virulent in this animal than HN878. Perhaps one reason for the differences seen between mice and guinea pigs could be the development of severe necrosis in multiple organs and the extensive extra-pulmonary dissemination observed in guinea pigs.

Morbidity and mortality in animal models of tuberculosis is due to the combined effect of pulmonary and extra-pulmonary pathology. While the lung is the first organ affected by *M. tuberculosis*, the infection spreads rapidly to extra-pulmonary sites by lymphatic and hematogenous dissemination. In most studies to date in which the lung pathology is considered, other organs are not often evaluated. In models relating survival to virulence, death of the animal can be due to multi-organ failure, not merely pulmonary disease. We show here that tuberculosis is a true multi-organ disease that spread to intestine, heart, adrenal glands among other organs. We also evaluated the lesion necrosis as a possible indicator of host susceptibility or the challenge-strain virulence. Necrosis plays a role in aerosol transmission of infectious bacilli^{21, 22} and resistance conferred in BCG-vaccination in guinea pigs is characterized by absence of necrosis in the primary granulomas of lungs and lymph nodes (2). The increased susceptibility of IFN- γ -deficient mice is also related to lesion necrosis²³. Acid-fast staining

revealed the bacilli to be present in the highest concentration in the necrotic core (data not shown).

Compared to H37Rv, the Erdman K01, CSU93 and HN878 infected animals consistently had more extensive necrosis in granulomas when the respective scores were compared in lungs, peribronchial lymph nodes, peripancreatic lymph nodes, spleen and liver (Table 1). Our results suggest that necrosis in the primary organs on day 30 of the infection, paralleled virulence of the different strains used in this study. Considering the importance of necrosis in tuberculosis pathogenesis, it is not surprising that degree of lesion necrosis paralleled strain virulence as determined by survival. H37Rv infected peribronchial lymph nodes showed less necrosis but extensive fibrosis compared to the other three strains indicating that while lymph node lesions in the H37Rv group began to somewhat resolve, the Erdman K01, CSU93 and HN878 groups had progressive necrosis with less evidence of lesion resolution.

Extra-pulmonary organ lesion scores of lymph nodes, spleen, liver, pancreas, heart, and to an extent the adrenal glands ranked consistently higher for the Erdman K01 and the two clinical strains compared to the laboratory strain H37Rv. Although the lesion scores did not accurately indicate the order of virulence, they were clearly higher for Erdman K01, CSU93 and HN878 groups compared to H37Rv group. The severity of disease in organs such as the pancreas, heart and to a lesser extent the adrenal glands indicated more extensive extra-pulmonary dissemination by Erdman K01, CSU93 and HN878 compared to H37Rv. The number of lesions in the heart directly correlated with the *M. tuberculosis*-strain virulence. These sites, which are rarely if ever examined, are of particular interest since extensive lesions in these organs can cause premature death before significant lung lesions develop and could therefore represent an important measure of virulence.

Another interesting finding was the development of severe inflammation and necrosis by day 30 of the infection in abdominal peripancreatic lymph nodes similar to those that directly drain the lungs (peribronchial lymph nodes). This finding along with pancreatic involvement and the fact that peripancreatic lymph nodes drain the stomach and anterior small intestine suggest that the bacilli reach the intestines directly and thus induce inflammation. This also suggests that bacilli might also spread through the gastro-intestinal route early in the course of the infection. This might occur either at the time of aerosol challenge or through swallowing the exudate cleared from the lung by mucocilliary action. It is generally thought that the lungs and their draining lymph nodes are the source of extra-pulmonary dissemination²⁴. However, our findings suggest that abdominal lymph nodes might also play a role in dissemination of the bacilli. Tuberculosis of pancreas and peripancreatic lymph nodes in humans is a rare condition²⁵. Nevertheless, the frequency of reports of pancreatic tuberculosis has increased in recent years along with the increase in the incidence of tuberculosis worldwide²⁶. As guinea pigs showed significant pathology in pancreas and peripancreatic lymph nodes, they may represent an appropriate animal model to study human pancreatic tuberculosis. The pancreatic involvement could be through retrograde spread of infection via pancreatic ducts or direct spread from peripancreatic lymph node due to their closer apposition.

We conclude that other than the survival studies, granuloma necrosis in pulmonary and extrapulmonary sites paralleled the virulence of *M. tuberculosis* strains. The incidence of heart lesions, which are indicative of more extensive extra-pulmonary dissemination, in fact paralleled well with virulence. In contrast, bacterial growth (CFUs) in the lungs, spleen or liver did not parallel with virulence as determined by survival suggesting that organ pathology is a better correlate of virulence than the bacterial growth. As pulmonary and extra-pulmonary organ pathology parallel well with virulence, the method described in detail in the “materials and methods” section can be used to evaluate the organ pathology of guinea pigs either on day 30 or day 60 of the infection in vaccine and drug evaluation studies as a measure of protection.

Since Erdman K01 strain caused more severe disseminated disease and resulted in shorter survival times, it would be ideal to use this strain as a target strain rather than H37Rv for challenge in vaccine and drug evaluation studies in guinea pigs or at least as an alternative strain for these studies.

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Figure 1.

Survival of Hartley guinea pigs infected with *M. tuberculosis* H37Rv (solid squares), Erdman K01 (solid diamond), CSU93 (solid triangle), and HN878 (solid inverted triangle) is compared. Median survival days are shown in Figure 1B. Survival results are from one experiment with 6 guinea pigs per group. Bacterial counts in the lungs (C), spleen (D) and liver (E) on day 30 from guinea pigs infected with a low dose of *M. tuberculosis* strain H37Rv, Erdman K01, CSU93 and HN878 were compared. Results are expressed as the average (n=5) of the bacterial load in each group expressed as Log₁₀CFU (± SE).

Figure 2.

Light photomicrographs of lungs (2A–D) and peribronchial lymphnodes (2E–H) from representative guinea pigs that were aerosol challenged with H37Rv (Figure 2A, 2E), Erdman K01 (Figure 2B, 2F), CSU93 (Figure 2C, 2G) and HN878 (Figure 2D, 2H) on day 30 are shown. On day 30, all three clinical isolates show extensive peribronchial and peribronchiolar involvement and more necrosis (arrows) in lungs compared to standard H37Rv strain. Necrosis in peribronchial lymph nodes (arrows) is relatively more extensive in clinical isolate groups compared to H37Rv (magnification 20x).

Figure 3.

Light photomicrographs of peripancreatic lymphnodes (3A–D) and spleens (3E–H) from representative guinea pigs that were aerosol challenged with H37Rv (Figure 3A, 3E), Erdman K01 (Figure 3B, 3F), CSU93 (Figure 3C, 3G) and HN878 (Figure 3D, 3H) on day 30 are shown. Necrosis in peripancreatic lymph nodes (arrows) is relatively more extensive in clinical isolate groups compared to H37Rv on day 30. Erdman K01 and HN878 show more necrosis (arrows) in spleens on day 30 (magnification 20x).

Figure 4.

Light photomicrographs of livers (4A–D) and pancreas (4E–H) from representative guinea pigs that were aerosol challenged with *Mtb* strain H37Rv (Figure 4A, 4E), Erdman K01 (Figure 4B, 4F), CSU93 (Figure 4C, 4G) and HN878 (Figure 4D, 4H) are shown. In liver, Erdman K01, CSU93 and HN878 groups show more lesions and necrosis (arrows) at the time of death than H37Rv group. Among all four groups, CSU93 shows most severe pancreatitis (arrows) (magnification 20x).

Figure 5.

Light photomicrographs of hearts (5A–D) and adrenal (5E–H) collected from representative guinea pigs that were aerosol challenged with *Mtb* strain H37Rv (Figure 5A, 5E), Erdman K01 (Figure 5B, 5F), CSU93 (Figure 5C, 5G) and HN878 (Figure 5D, 5H) at the time of their death are shown. Among all four groups, Erdman K01 shows most severe myocarditis (arrows). Erdman K01 and HN878 show prominent adrenalitis (arrows) compared to the other two strains (magnification 20x).

Table 1

Mean total lesion scores

The lesion scores of lungs, peribronchial lymph nodes (PBLN), peripancreatic lymph nodes (PPLN), spleen, liver, pancreas, adrenal and heart were calculated as described on materials and methods for all the four groups on day 30 of the infection and at the time of death. The scores are presented as total mean lesion values.

	Lungs	PBLN	PPLN	Spleen	Liver	Pancreas	Adrenal	Heart
Day 30								
H37Rv	6.25	9.5	9	4	3.5	0	0	0.25
ErdK01	9	10.25	10	7.75	3.25	0	0	3
CSU93	8	10.5	11	4.75	3.5	0	0	1.25
HN878	9.25	10	10.75	6.25	4	0	0	2
At death								
H37Rv	11	10	12.5	7.67	6.7	1.4	1.6	0.3
ErdK01	14.5	11.5	13.67	9.75	8.75	2.33	2.5	3.8
CSU93	12.3	10.75	12.67	10	7.8	3.8	1	2.8
HN878	13.1	14.4	15	8.6	9	2	3	2.8

Table 2

Mean necrosis and mineralization scores

The necrosis and mineralization scores of lungs, peribronchial lymph nodes (PBLN), peripancreatic lymph nodes (PPLN), spleen and liver were calculated as described on materials and methods for all the four groups on day 30 of the infection and at the time of death and are presented as mean values in Table 2.

Organs	Lungs										PBLN									
	Day 30					At death					Day 30					At death				
	a	b	c	d		a	b	c	d		a	b	c	d		a	b	c	d	
Necrosis	1.8	3.5	3.3	4.0	2.5	4.0	3.3	3.5	3.5		3.8	4.8	4.5	4.0	3.0	4.0	4.0	3.5	4.4	
Mineralization	0	0	0	0	1.7	3.0	1.8	2.7	2.7		0	0	0	0	0	0	0	0	3.0	
Organs	PPLN										Spleen									
	Day 30					At death					Day 30					At death				
	a	b	c	d		a	b	c	d		a	b	c	d		a	b	c	d	
Necrosis	3.0	4.0	5.0	4.8	3.7	4.0	4.0	4.4	4.4		1.5	3.3	2.8	2.8	2.2	2.5	2.5	2.8	2.6	
Mineralization	0	0	0	0	0.2	0.5	1.2	2.4	2.4		0	0	0	0	0	0	0	0.4	0.7	
Organs	Liver																			
	Day 30					At death														
	a	b	c	d		a	b	c	d											
Necrosis	2.8	2.3	2.3	2.8	2.7	3.5	3.3	3.7	3.7											
Mineralization	0.4	0	0	0	0	0.5	0	0	0											