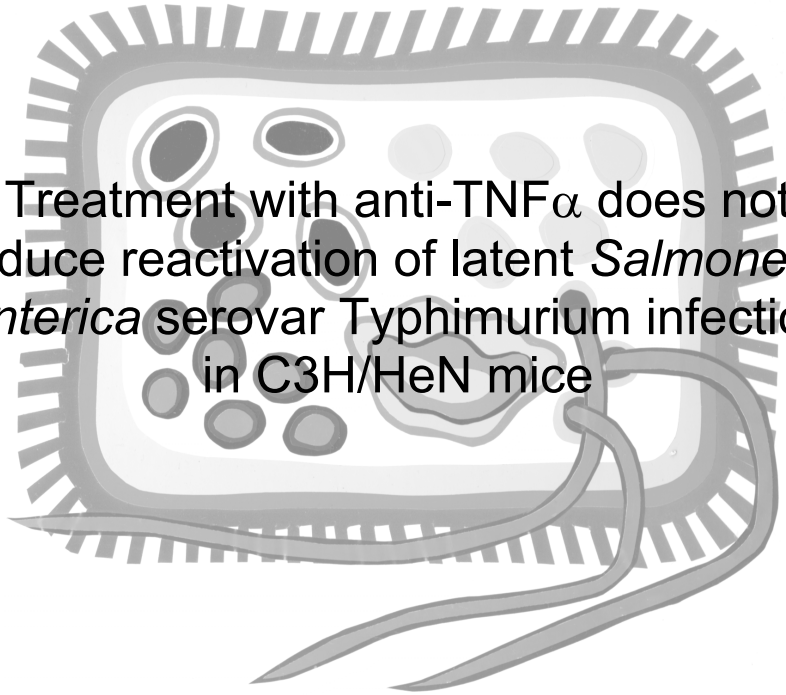
Salmonella typhimurium and its host : host-pathogen cross-talk, immune evasion, and persistence

Diepen, A. van

chapter three



Treatment with anti-TNFα does not induce reactivation of latent *Salmonella enterica* serovar Typhimurium infection in C3H/HeN mice

Angela van Diepen,[1] Cerithsa Martina,[1] Riny Janssen,[1] and Jaap T. van Dissel[1]

Department of Infectious Diseases, Leiden University Medical Center, PO Box 9600, 2300 RC Leiden, The Netherlands.[1]

Submitted for publication

Abstract

TNFα is a tightly regulated non-specific effector molecule that has pro-inflammatory and immuno-regulatory effects. The action of TNFα appears to be unopposed and results in damage to tissue, e.g. cartilage or bowel mucosa. In some inflammatory disorders, TNFα is induced, but fails to decrease leading to high amounts of this potent and damaging pro-inflammatory cytokine. Nowadays, patients suffering from these disorders can be successfully treated with the TNFα blocking agents Infliximab or Ethanercept. However, this treatment carries an increased risk of manifest infection with *Mycobacteria* and might lead to reactivation of latent infections, as has been described for intracellular pathogens such as *Mycobacteria*.

Reactivation of latent *Salmonella* infection has been described for patients with an impaired immune system due to medication or underlying disease, and a role for IFNγ and $CD4^+$ T cells during the phase of persistency of *Salmonella* has been described. Since TNFα plays an important role in defense to primary *Salmonella* infections, we tested whether treatment with TNFα neutralizing agents may lead to reactivation of a latent *Salmonella* infection. In the C3H/HeN mouse model of latent *S. enterica* serovar Typhimurium infection, in contrast to previous findings on reactivation after CD4+ T cell depletion or anti-IFNγ treatment, neutralization of TNFα did not lead to reactivation and suggests only a minor role for TNFα during the phase of latency.

Introduction

TNFα is a non-specific effector molecule that is mainly produced by phagocytes (neutrophils and macrophages) and that has pro-inflammatory and immuno-regulatory effects. TNFα can act both as a membrane-associated protein and as a soluble cytokine after cleavage from the cell surface by the TNFα converting enzyme (2, 16, 20). Both soluble and membrane-bound TNFα are able to bind to the TNF receptor (TNFR) 1 as well as to TNFR2. Soluble TNFα preferentially binds to and activates the TNFR1, while TNFR2 is mainly engaged and activated by the membrane-bound form of TNFα. Activation of TNFR1 leads to a whole range of cellular and tissue responses such as induction of cytokine and chemokines production, MHC class I and II expression, cell adhesion molecule expression, inhibition of cell growth, apoptosis, tissue repair and damage, neurotoxicity and neuroprotection. TNFR2 activation leads to thymocyte proliferation, skin necrosis, and T-cell proliferation and apoptosis. TNF receptors are expressed on the surface of most cell types (30), so TNFα exerts its effects on almost every cell and organ within the body. Therefore, the production of TNFα is strictly regulated during infection. TNFα production is induced rapidly, but also degraded at a high rate.

In some inflammatory diseases such as rheumatoid arthritis and inflammatory bowel disease, TNFα is induced, but fails to decrease. In these patients, the regulation of TNFα appears to be disturbed leading to the production of high amounts of this potent but damaging pro-inflammatory cytokine. Recently, new therapeutics have been developed to lessen the damage induced by TNFα , i.e. Infliximab (Remicade®, Centocor Inc) and Ethanercept (Enbrel®, Wyeth/Immunex), which are TNFα-blocking agents that are presently applied in the treatment of patients with rheumatoid arthritis and Crohn's disease. This treatment is highly beneficial for these patients. However, an important disadvantage of treatment with neutralizing antibodies to TNFα is the increased susceptibility to infection with intracellular bacteria. By its ability to activate macrophages to enhanced microbicidal activity and role in the formation of granulomas, TNFα has been shown to play a central role in immunity to bacterial infections with *Mycobacteria* (reviewed in (1, 9)), *Listeria moncytogenes* (4), *Salmonella* (22) and some other bacterial pathogens (6-8, 11, 12, 15, 21, 23, 25).

Besides the increased susceptibility to primary infection with intracellular bacteria, another complication of treatment with anti-TNFα is reactivation of latent infection with such bacteria, as has been described for *Mycobacteria.* Keane et al. reported 70 cases of tuberculosis (TB) among ~147,000 patients treated with TNFα blocking agents world-wide (14). The early onset after start of anti-TNFα treatment and low background incidences of TB is suggestive of reactivation rather than primary infection (14) and shows a role for TNFα in controlling latent infection with *Mycobacteri*a. In line with these observations, it has been speculated that treatment with neutralizing antibodies to TNFα could lead to reactivation of latent *Salmonella* infection as well.

Reactivation of infection of non-typhoidal *Salmonella* strains that had persisted within the host have been described for patients with an impaired functioning immune system

such as HIV-infected individuals (5, 10, 13), with Interleukin 12 receptor β1 deficiency (26), or patients with hematologic malignancies who underwent total body irradiation or who received a solid organ transplant and were treated with glucocorticoids or other immunosuppressive therapy.

Recently, we have shown that $CD4^+$ T cells play an important role during the phase of latency in C3H/HeN (Ity^r) mice and that depletion of the $CD4^+$ T cells resulted in reactivation of latent infection (29). Monack et al. have shown that neutralization of IFNγ resulted in reactivation of latent *S. enterica* serovar Typhimurium as well (19). $CD4^+$ T cells and IFNγ are important for activation of macrophages by inducing the release of TNFα, which via an autocrine loop acts on the macrophages' TNF receptor and leads to the formation of an angry macrophage expressing enhanced bacterial killing. Thus, given the pivotal role that TNFα plays in the cellular immune response, some problems with reactivation of persistent infections were to be expected in those receiving treatment with anti- TNFα.

The role of TNFα in mice during a primary infection with *Salmonella* enterica serovar Typhimurium has been known for years (18), however, its role in controlling latent infection is unclear. Therein, we used the mouse model for latent *S. enterica* serovar Typhimurium infection as described in detail recently (29) to address the question whether reactivation of the persistent *S. enterica* serovar Typhimurium infection is induced upon neutralization of TNFα by treating the mice with Ethanercept after full recovery from a primary infection with *S. enterica* serovar Typhimurium.

Materials and Methods

Mice. Six- to 8-week-old female *Salmonella*-resistant (Ity^r) C3H/HeN mice were purchased from Harlan (Horst, The Netherlands). Mice were maintained under standard conditions according to institutional guidelines. Water and food were given ad libitum. Experiments were approved by the local Animal Ethical Committee.

Bacterial strains and growth conditions. For the infection experiments wild-type *S. enterica* serovar Typhimurium 14028s was used. Single colonies of the different strains were grown in Luria-Bertani (LB) medium (10 mg of tryptone, 5 mg of yeast extract, and 10 mg of NaCl/ml) at 37°C while being shaken (225 rpm). For the in vivo experiments, the overnight cultures were diluted in fresh LB medium and grown to the end of log phase and were then washed and diluted in sterile PBS. The CFU in the inoculum were determined by plating serial dilutions.

In vitro infection experiment. RAW264.7 cells were seeded in a 24-wells plate at a density of 1×10^5 cells per well and allowed to adhere at 37°C in RPMI medium supplemented with 10% fetal calf serum. The cells were left unaffected or were incubated with 100 U recombinant IFNγ (Pharmingen International) and/or 0.5 mg anti-TNFα

antibodies (Enbrel) for 18 h prior to infection. Bacteria were washed in PBS and were added to the cells at a 10:1 multiplicity of infection. The bacteria were spun onto the cell by centrifugation for 30 min at 270 × g. Cells were incubated for 30 min at 37°C and 5% CO_2 to allow bacterial endocytosis. After washing the cells with PBS, medium containing 100 μg/ml gentamicin was added and incubated at 37°C for another 30 min to kill the extracellular bacteria. The cells were then washed again. This point was designated time point zero. Next, medium containing 10 μg/ml gentamicin was added to the cells to kill any remaining extracellular bacteria and prevent reinfection. At 0, 24, and 48 hours the cells were lysed in 1 ml H_2O and serial dilutions were made to determine the number of bacteria.

In vivo *S. enterica* serovar Typhimurium infection. Mice were inoculated subcutaneously in the flanks with 3×10^4 CFU *S. typhimurium* 14028s. For each group on each time point 3-4 mice were used. Fecal samples were taken and mice were sacrificed by carbon dioxide inhalation. Blood was obtained immediately by cardiac puncture. Part of the blood was prevented to coagulate by adding 40 U heparin/ml and another part was used to obtain serum. To determine the bacterial load within spleens, livers, mesenteric and inguinal lymph nodes, these organs were aseptically removed and single cell suspensions were prepared by using sterile 70-µm-mesh-size cell strainers (Falcon). Cells were pelleted by centrifugation for 10 min and were lysed in distilled water. The bacterial number per organ was determined bacteriologically by plating serial dilutions. The lowest number of bacteria that can be detected in this way is 30 CFU for the spleens and lymph nodes and 100 CFU for the livers. The feces was weighed and suspended in PBS and was plated on selective (SS) agar to determine the number of *S. enterica* serovar Typhimurium.

Leukocyte count and blood cell differentiation. The number of peripheral blood leukocytes was determined by counting the number of nucleated cells in the heparinized blood. In addition, we used 5 μl blood to make blood smears for the differentiation of the blood cells. Blood smears were fixed in methanol for 15 min and were stained with Giemsa for 30 min. In the blood smears the relative percentages of the different types of cells were determined. Next, the number of lymphocytes, monocytes, and PMN present in the blood on the different time points was calculated.

Treatment with neutralizing TNFα antibodies and dexamethasone. Immune intervention was carried out either by intraperitoneal injection of the mice with with 300 μg Ethanercept (a concentration known to have an effect in mice, personal communication with R. Flierman, Dpt. of Rheumatology, LUMC, The Netherlands) or 6 mg/kg dexamethasone on a time point when bacteria could no longer be detected bacteriologically within livers, spleens, and inguinal lymph nodes. Mice received a second and a third injection with 300 μg of Ethanercept or 6 mg/kg dexamethasone on day two and four after the first injection.

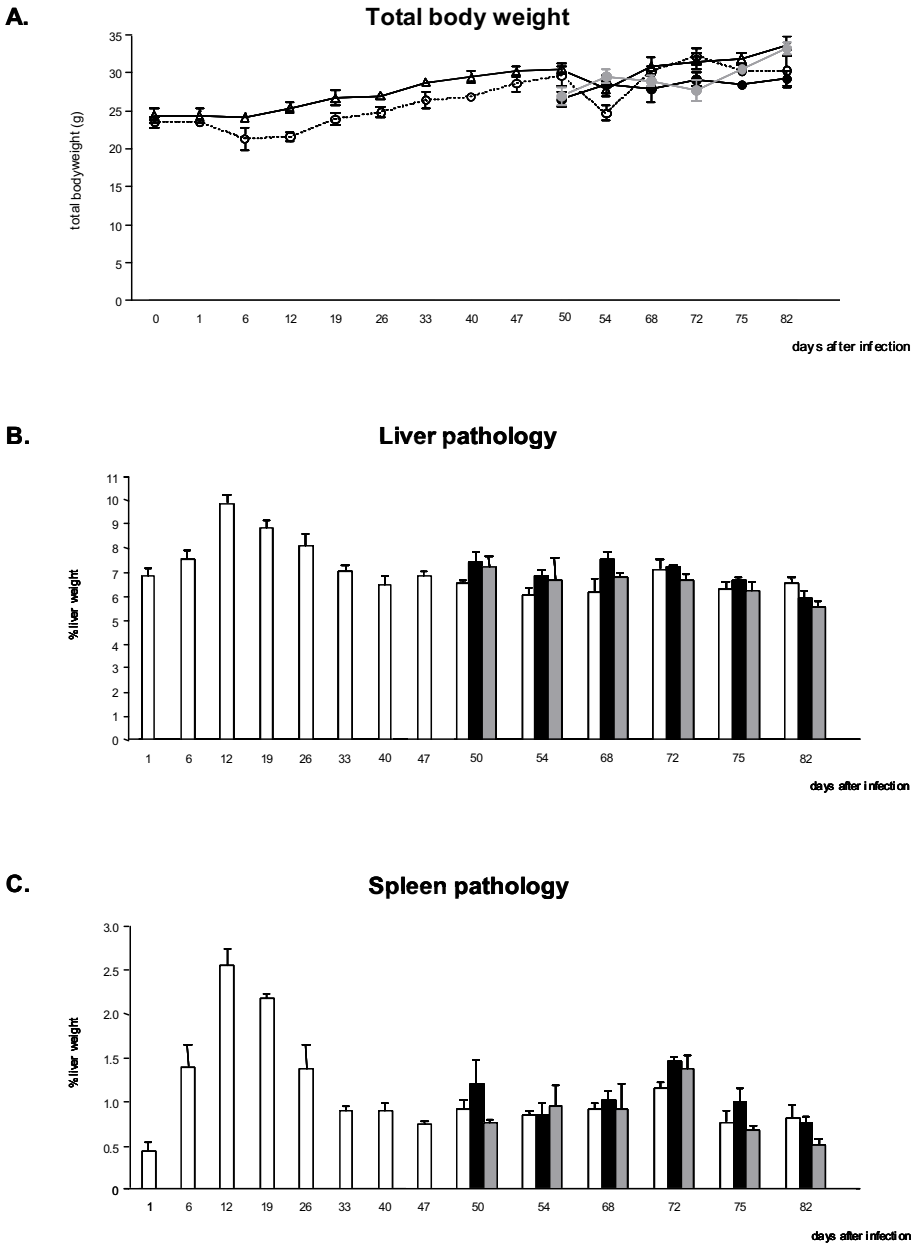
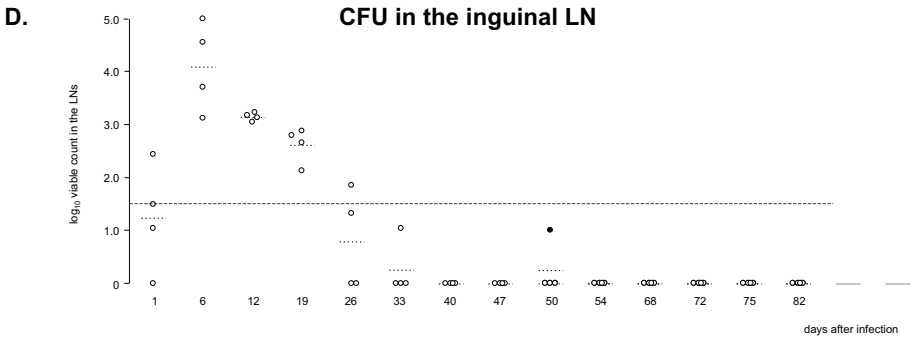
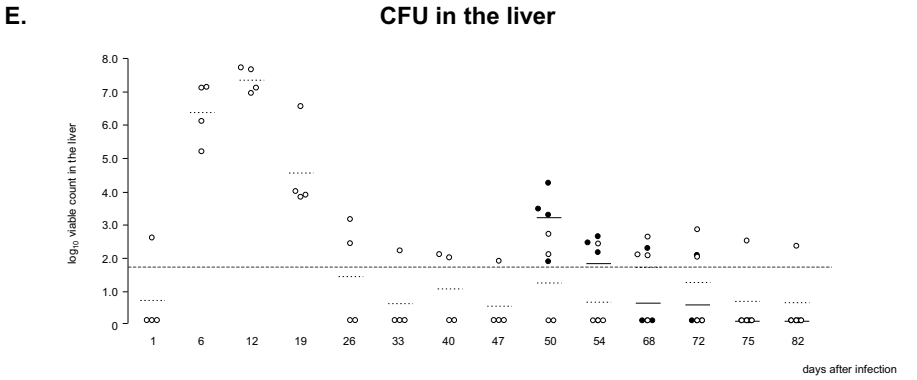


Figure 1. Total body weight (A), liver and spleen pathology (B and C) and bacterial numbers within the lymph nodes (D), livers (E), and spleens (F) of C3H/HeN (Ity^r) mice. On day 0, C3H/HeN (Ity^r) mice were injected subcutaneously in the flanks with ~3×10^4 CFU *S. enterica* serovar Typhimurium 14028s. The dose was confirmed by plating serial dilutions of the inoculum. Four age-matched mice were not infected and served as weight controls (white triangles). After full recovery from the primary infection, the mice were injected i.p. with PBS (white dots and bars), anti-TNFα antibodies (black dots and bars), or dexamethasone (grey dots and bars).





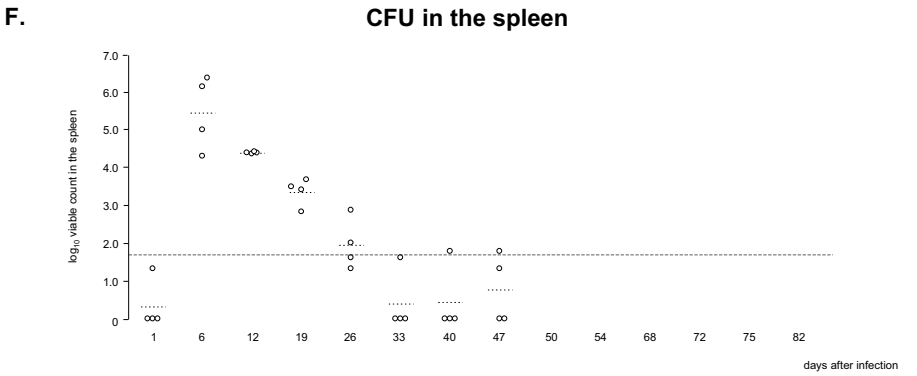


Figure 1 continued. At the indicated time points, the livers, spleens, and lymph nodes were aseptically removed and weighed. The pathology in the livers and spleens was calculated as the percentage organ weight of the total body weight. The viable count within the organs was determined by making lysates and plating serial dilutions of the lysates (n=3-4 per group). The results are expressed as $\log_{10}$ viable counts (means $\pm$ standard deviations). The gray dashed lines represent the detection limit of the microbiological method.

Detection of antibodies raised against *S. enterica* serovar Typhimurium. Induction of *S. enterica* serovar Typhimurium-specific antibodies was determined in a whole cell ELISA as described in (28). Maxisorp plates (Nunc) were coated with whole *S. enterica* serovar Typhimurium 14028s and after blocking, serial dilutions of the sera were added to the wells. Sera from naïve mice were included as a control. The wavelength absorbance was measured at 490 nm using an ELISA plate reader (VICTOR^2 1420 multilabel counter, PerkinElmer Life and Analytical Sciences). Titers are defined as the dilution for which:

$$OD_{450} \text{ (sample)} > OD_{450} \text{ (naïve serum)} + 2 \times \text{standard deviation.}$$

Statistical analysis. For comparison between treatments we used Student's *t* tests and a P value <0.05 was considered significant.

Results and Discussion

No reactivation following neutralization of TNFα. Mice were inoculated subcutaneously in the flanks with 3×10^4 CFU *S. enterica* serovar Typhimurium 14028s. After full recovery from the primary infection, the mice were injected with neutralizing antibodies to TNFα. The mice lost weight during the primary infection reaching a nadir between day 6 and 12 and gained weight during the next few weeks following a course comparable to that of the weight controls (Fig. 1A). The mice showed a primary infection reaching bacterial loads up to 1×10^6 and 2.5×10^4 CFU for the spleen and lymph nodes respectively on day 6 and 1.5×10^7 for the liver on day 12.

On day 48, 50, and 54, when the bacterial numbers in the organs were reduced to below the detection limit, the mice were treated with anti-TNFα antibodies. Infection controls were injected with equal volumes of PBS. None of the mice showed signs of illness and they all gained weight (Fig. 1A) and showed no consistent change in liver and spleen weight (Fig. 1B and 1C). Anti-TNFα treatment had no effect on the bacterial numbers in the organs (Fig. 1D-F), although treatment with anti-TNFα antibodies did induce a transient increase in bacterial numbers in the livers (Fig. 1E). In both groups, the mice showed detectable amounts of bacteria in the livers and spleens (Fig. 1B and 1C), but the averages were around or below the detection limit and no increase in bacterial numbers could be observed. In the feces on the other hand, we were able to detect bacteria on the later timepoints in these groups of mice. In our reactivation model we accepted that some of the control mice still showed some low number of bacteria in the organs, just above the limit of detection. Otherwise, we would have needed many more animals to find only a few in which *S. typhimurium* persisted and reactivated upon treatment.

Leukocyte counts in the blood. On each time point after infection and immune intervention, the number of leukocytes in the blood was determined and the types of blood

cells were differentiated using Giemsa stained blood smears (Table 1). During the growth phase of the primary infection, the number of leukocytes increased which was mainly attributable to the increase in the number of granulocytes (Table 1). During recovery from the infection, the leukocyte counts declined and eventually stabilized. Treatment of the mice with neutralizing antibodies to TNFα did not result in a change in the number of leukocytes, nor in the numbers of lymphocytes, granulocytes, and monocytes (Table 1).

Anti-*Salmonella* IgG antibodies in the serum of *S. enterica* serovar Typhimurium infected mice. From each mouse on each timepoint, serum was collected to determine the anti-*Salmonella* IgG antibody titer to the pathogen using a whole cell ELISA. Between days 6 and 12, when the primary infection peaked in the organs, the mice started producing antibodies to *S. enterica* serovar Typhimurium 14028s (Fig. 2). The antibody titers increased further to a $\log_3$ dilution factor of around 8 (Fig. 2). The mice that were treated with anti-TNFα antibodies had serum antibody levels that were similar, or slightly lower on days 50 and 5 to those of the infection controls so lack of reactivation could not be explained by altered antibody production in these mice.

In vitro infection of IFNγ stimulated RAW264.7 macrophages. To examine the role of TNFα in vitro in RAW264.7 macrophages, we performed in vitro infection experiments in which we stimulated the cells overnight with 100 U IFNγ and infected the cells as described in Materials & Methods. Just prior to infection, cells were treated with 10 μg/ml Anti-TNFα antibodies or were left untreated. At timepoint zero and after 24 h the number of intracellular bacteria was determined. In the untreated cells, the bacteria were able to grow out within 24 h to 1.5×10^6 CFU. Pre-treatment of the cells with 100 U IFNγ resulted in no outgrowth after 24 h, while treatment with anti-TNFα antibodies had no effect. When cells were activated with IFNγ and anti-TNFα treatment had no effect on the number of bacteria (Fig. 3), indicating that anti-TNFγ treatment cannot neutralize the effect of IFNγ.

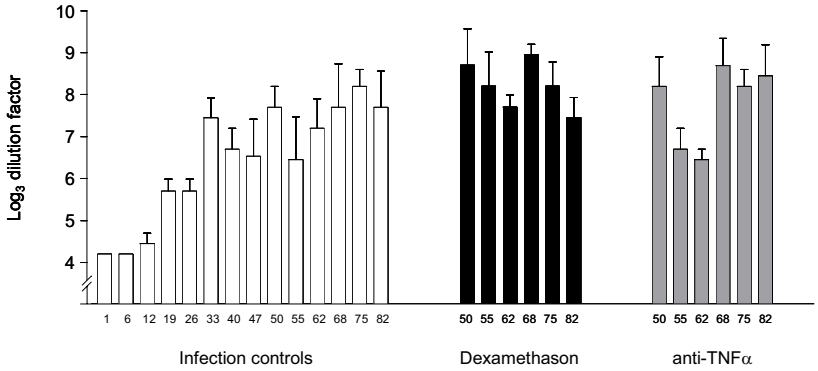


Figure 2. Anti-*Salmonella* IgG antibodies in the serum of *S. enterica* serovar Typhimurium infected mice that received no further treatment (white bars), were treated with anti-TNFα antibodies (black bars), or were treated with dexamethasone (grey bars). Titers are defined as the OD_{450}(serum sample) > OD_{450}(naïve serum).

Table 1. Number of leukocytes, lymphocytes, monocytes, and granulocytes in the blood[a]

day	treatment	leukocytes ($n \times 10^5$)	lymphocytes ($n \times 10^5$)	monocytes ($n \times 10^5$)	granulocytes ($n \times 10^5$)
1	---	64.3 ± 9.1	42.3 ± 5.6	4.8 ± 0.8	17.2 ± 8.9
5	---	73.8 ± 54.0	28.0 ± 17.9	9.8 ± 6.8	35.9 ± 7.3
12	---	77.2 ± 15.2	27.2 ± 10.9	9.9 ± 5.4	40.1 ± 13.6
19	---	95.3 ± 23.8	38.2 ± 8.7	10.6 ± 6.3	46.5 ± 13.1
26	---	80.8 ± 34.7	44.9 ± 16.1	5.1 ± 3.1	30.8 ± 11.7
33	---	46.5 ± 4.2	32.0 ± 3.2	2.5 ± 1.8	12.0 ± 29.2
40	---	120.0 ± 14.1	90.8 ± 4.0	6.2 ± 3.3	23.1 ± 6.9
47	---	104.3 ± 23.8	76.4 ± 20.0	5.3 ± 1.6	22.6 ± 5.4
50	---	117.7 ± 38.7	80.1 ± 22.4	5.4 ± 2.6	32.1 ± 17.1
50	αTNF[b]	155.0 ± 28.9	89.5 ± 36.8	12.0 ± 6.8	53.5 ± 23.1
50	dex[c]	172.5 ± 38.6	100.5 ± 17.6	10.3 ± 3.5	61.7 ± 19.3
54	---	72.3 ± 16.3	48.5 ± 13.5	5.1 ± 2.4	18.7 ± 8.4
54	αTNF	62.0 ± 16.7	37.4 ± 6.1	6.3 ± 2.8	18.4 ± 8.6
54	dex	50.3 ± 7.3	15.8 ± 5.5	7.1 ± 2.6	27.4 ± 6.4
68	---	57.0 ± 13.0	30.8 ± 6.4	4.4 ± 2.0	21.8 ± 8.0
68	αTNF	66.5 ± 26.6	38.6 ± 15.8	4.0 ± 2.0	23.9 ± 9.7
68	dex	38.3 ± 12.8	18.4 ± 4.1	3.3 ± 1.4	16.5 ± 8.4
72	---	58.3 ± 15.5	37.1 ± 9.3	4.0 ± 1.3	17.1 ± 6.1
72	αTNF	66.5 ± 7.3	38.7 ± 6.7	4.0 ± 1.1	23.8 ± 6.0
72	dex	48.5 ± 17.7	22.7 ± 6.0	2.2 ± 0.3	23.6 ± 12.6
75	---	135.0 ± 30.0	94.2 ± 9.3	5.6 ± 3.0	35.2 ± 19.7
75	αTNF	187.5 ± 42.7	106.8 ± 6.7	10.9 ± 8.1	69.8 ± 30.8
75	dex	142.5 ± 41.1	92.1 ± 6.0	5.9 ± 0.9	44.5 ± 11.8
82	---	79.0 ± 8.5	52.4 ± 11.3	1.8 ± 0.5	24.8 ± 12.1
82	αTNF	87.0 ± 15.0	62.3 ± 19.9	1.6 ± 0.3	23.1 ± 5.1
82	dex	57.5 ± 14.0	37.6 ± 29.1	1.6 ± 0.5	18.2 ± 6.8

[a] values are mean numbers/ml ± stdev
[b] mice that were treated with anti-TNFα
[c] mice that were treated with dexamethasone

Dexamethasone treatment of mice during latency. In the experiment shown in Figure 1 we have also treated mice with the glucocorticoid dexamethasone. Glucocorticoids have immunosuppressive effects through the inhibition of several immune functions, including chemotaxis, phagocytosis, and cytotoxicity, and by the down-regulation of cytokine gene expression, including IL-I, IL-2, IL-6, IFN-γ, and TNF-α (3). Glucocorticoids are known to inhibit neutrophil infiltration at inflammatory sites, thereby inhibiting neutrophil-mediated killing via mechanisms that are no completely understood yet. Glucocorticoids exert an anti-inflammatory effect and downregulate the expression of ICAM-1, which is constitutively expressed on neutrophils and vascular endothelial cells and is upregulated by inflammatory cytokines (17, 24, 27) such as TNFα. Treatment with dexamethasone (and other glucocorticoids) might promote reactivation of latent *S. typhimurium* infection by inhibiting the neutrophil-mediated killing. Treatment with dexamethasone resulted in a slight increase in the number of granulocytes and moderate decrease in lymphocyte number (Table 1). Dexamethasone treatment did not have an effect on body weight and hepatosplenomegaly (Fig. 1A, B, and C), nor did it induce reactivation of the latent *S. enterica* serovar Typhimurium infection in the organs (Fig. 1D, E, and F). Also in these mice, antibody production was induced to a similar extent as in the infection controls (Fig. 2).

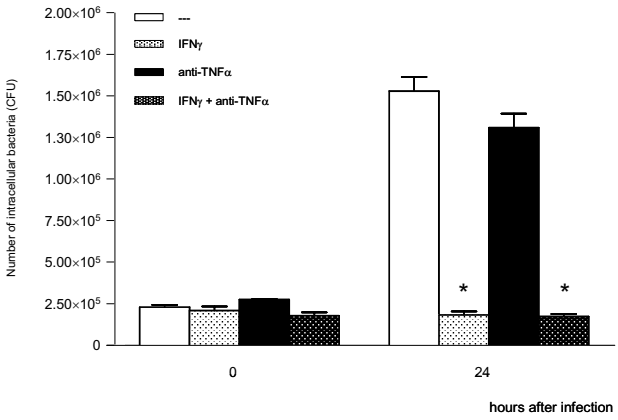


Figure 3. Intracellular *S. enterica* serovar Typhimurium 14028s in RAW264.7 macrophages. The cells were left untreated (white bars), stimulated with IFNγ (white bars with black dots), not stimulated but treated with anti-TNFα antibodies (black bars) or stimulated with IFNγ and treated with anti-TNFα antibodies (black bars with white dots). All cells were challenged with *S. enterica* serovar Typhimurium 14028s as described in Materials and Methods and the numbers of intracellular bacteria were determined at 24 h after challenge. Asterisks indicate that the number of intracellular bacteria is significantly different from that of wild-type *S. enterica* serovar Typhimurium 14028s. Data from a representative experiment are shown.

Concluding remarks. The main finding of this study is that neutralization of TNFα did not result in reactivation of latent *S. enterica* serovar typhimurium infection in C3H/HeN mice. This is in sharp contrast to the many reports of reactivating Mycobacterial infections after neutralization of TNFα. Both species are intracellular pathogens that preferentially invade mononuclear cells and have developed mechanisms to reside within a host without being noticed by the host immune system and are much alike. For both *Salmonella* and *Mycobacteria* an important role has been ascribed to TNFα in preventing or dealing with a primary infection. Regarding recurrent infections, there are sharp differences. For

Mycobacteria, recurrence of latent infections due to anti-TNFα have been described several times now, while this is not the case for *Salmonella*. Recurrent infections with the same *Salmonella* strain have been described for patients with immune disorders such as HIV and recently a role for $CD4^+$ T cells and IFNγ in preventing recurrence of a latent infection has been described. This strongly suggests that TNFα plays only a modest role in preventing reactivation while IFNγ and $CD4^+$ T cells play a much more important role.

References

1. **Arend, S. M., F. C. Breedveld, and J. T. van Dissel.** 2003. TNF-alpha blockade and tuberculosis: better look before you leap. Neth. J. Med. **61:**111-119.
2. **Black, R. A., C. T. Rauch, C. J. Kozlosky, J. J. Peschon, J. L. Slack, M. F. Wolfson, B. J. Castner, K. L. Stocking, P. Reddy, S. Srinivasan, N. Nelson, N. Boiani, K. A. Schooley, M. Gerhart, R. Davis, J. N. Fitzner, R. S. Johnson, R. J. Paxton, C. J. March, and D. P. Cerretti.** 1997. A metalloproteinase disintegrin that releases tumor-necrosis factor-alpha from cells. Nature **385:**729-733.
3. **Boumpas, D. T., F. Paliogianni, E. D. Anastassiou, and J. E. Balow.** 1991. Glucocorticosteroid action on the immune system: molecular and cellular aspects. Clin. Exp. Rheumatol. **9:**413-423.
4. **Bowie, V. L., K. A. Snella, A. S. Gopalachar, and P. Bharadwaj.** 2004. *Listeria* meningitis associated with infliximab. Ann. Pharmacother. **38:**58-61.
5. **Burckhardt, B., P. Sendi, D. Pfluger, W. Zimmerli, R. Nuesch, H. C. Bucher, J. Drewe, N. Gyr, and M. Battegay.** 1999. Rare AIDS-defining diseases in the Swiss HIV Cohort Study. Eur. J. Clin. Microbiol. Infect. Dis. **18:**399-402.
6. **Everest, P., M. Roberts, and G. Dougan.** 1998. Susceptibility to *Salmonella typhimurium* infection and effectiveness of vaccination in mice deficient in the tumor necrosis factor alpha p55 receptor. Infect. Immun. **66:**3355-3364.
7. **Flynn, J. L., M. M. Goldstein, J. Chan, K. J. Triebold, K. Pfeffer, C. J. Lowenstein, R. Schreiber, T. W. Mak, and B. R. Bloom.** 1995. Tumor necrosis factor-alpha is required in the protective immune response against *Mycobacterium tuberculosis* in mice. Immunity. **2:**561-572.
8. **Garcia, I., Y. Miyazaki, G. Marchal, W. Lesslauer, and P. Vassalli.** 1997. High sensitivity of transgenic mice expressing soluble TNFR1 fusion protein to mycobacterial infections: synergistic action of TNF and IFN-gamma in the differentiation of protective granulomas. Eur. J. Immunol. **27:**3182-3190.
9. **Gardam, M. A., E. C. Keystone, R. Menzies, S. Manners, E. Skamene, R. Long, and D. C. Vinh.** 2003. Anti-tumour necrosis factor agents and tuberculosis risk: mechanisms of action and clinical management. Lancet Infect. Dis. **3:**148-155.
10. **Gordon, M. A., H. T. Banda, M. Gondwe, S. B. Gordon, M. J. Boeree, A. L. Walsh, J. E. Corkill, C. A. Hart, C. F. Gilks, and M. E. Molyneux.** 2002. Non-typhoidal *Salmonella* bacteraemia among HIV-infected Malawian adults: high mortality and frequent recrudescence. AIDS **16:**1633-1641.
11. **Havell, E. A.** 1989. Evidence that tumor necrosis factor has an important role in antibacterial resistance. J. Immunol. **143:**2894-2899.
12. **Hultgren, O., H. P. Eugster, J. D. Sedgwick, H. Korner, and A. Tarkowski.** 1998. TNF/lymphotoxin-alpha double-mutant mice resist septic arthritis but display increased mortality in response to *Staphylococcus aureus*. J. Immunol. **161:**5937-5942.
13. **Hung, C. C., S. M. Hsieh, C. F. Hsiao, M. Y. Chen, and W. H. Sheng.** 2001. Risk of recurrent non-typhoid *Salmonella* bacteraemia after early discontinuation of ciprofloxacin as secondary prophylaxis in AIDS patients in the era of highly active antiretroviral therapy. AIDS **15:**645-647.
14. **Keane, J., S. Gershon, R. P. Wise, E. Mirabile-Levens, J. Kasznica, W. D. Schwieterman, J. N. Siegel, and M. M. Braun.** 2001. Tuberculosis associated with infliximab, a tumor necrosis factor alpha-neutralizing agent. N. Engl. J. Med. **345:**1098-1104.
15. **Kindler, V., A. P. Sappino, G. E. Grau, P. F. Piguet, and P. Vassalli.** 1989. The inducing role of tumor necrosis factor in the development of bactericidal granulomas during BCG infection. Cell **56:**731-740.
16. **Kriegler, M., C. Perez, K. DeFay, I. Albert, and S. D. Lu.** 1988. A novel form of TNF/cachectin is a cell surface cytotoxic transmembrane protein: ramifications for the complex physiology of TNF. Cell **53:**45-53.
17. **Mandi, Y., Z. Nagy, I. Ocsovski, and G. Farkas.** 1997. Effects of tumor necrosis factor and pentoxifylline on ICAM-1 expression on human polymorphonuclear granulocytes. Int. Arch. Allergy Immunol. **114:**329-335.
18. **Mastroeni, P., B. Villarreal-Ramos, and C. E. Hormaeche.** 1993. Effect of late administration of anti-TNF alpha antibodies on a *Salmonella* infection in the mouse model. Microb. Pathog. **14:**473-480.
19. **Monack, D. M., D. M. Bouley, and S. Falkow.** 2004. *Salmonella typhimurium* Persists within Macrophages in the Mesenteric Lymph Nodes of Chronically Infected Nramp1+/+ Mice and Can Be Reactivated by IFN{gamma} Neutralization. J. Exp. Med. **199:**231-241.
20. **Moss, M. L., S. L. Jin, M. E. Milla, D. M. Bickett, W. Burkhart, H. L. Carter, W. J. Chen, W. C. Clay, J. R. Didsbury, D. Hassler, C. R. Hoffman, T. A. Kost, M. H. Lambert, M. A. Leesnitzer, P. McCauley, G.**

McGeehan, J. Mitchell, M. Moyer, G. Pahel, W. Rocque, L. K. Overton, F. Schoenen, T. Seaton, J. L. Su, J. D. Becherer, and . 1997. Cloning of a disintegrin metalloproteinase that processes precursor tumour-necrosis factor-alpha. Nature **385:**733-736.

21. **Nakane, A., T. Minagawa, and K. Kato.** 1988. Endogenous tumor necrosis factor (cachectin) is essential to host resistance against *Listeria monocytogenes* infection. Infect. Immun. **56:**2563-2569.
22. **Netea, M. G., T. Radstake, L. A. Joosten, J. W. Van der Meer, P. Barrera, and B. J. Kullberg.** 2003. *Salmonella* septicemia in rheumatoid arthritis patients receiving anti-tumor necrosis factor therapy: association with decreased interferon-gamma production and Toll-like receptor 4 expression. Arthritis Rheum. **48:**1853-1857.
23. **Senaldi, G., S. Yin, C. L. Shaklee, P. F. Piguet, T. W. Mak, and T. R. Ulich.** 1996. *Corynebacterium parvum*- and *Mycobacterium bovis* bacillus Calmette-Guerin-induced granuloma formation is inhibited in TNF receptor I (TNF-RI) knockout mice and by treatment with soluble TNF-RI. J. Immunol. **157:**5022-5026.
24. **Shirasaki, H., K. Watanabe, E. Kanaizumi, J. Sato, N. Konno, S. Narita, and T. Himi.** 2004. Effect of glucocorticosteroids on tumour necrosis factor-alpha-induced intercellular adhesion molecule-1 expression in cultured primary human nasal epithelial cells. Clin. Exp. Allergy **34:**945-951.
25. **Skerrett, S. J., T. R. Martin, E. Y. Chi, J. J. Peschon, K. M. Mohler, and C. B. Wilson.** 1999. Role of the type 1 TNF receptor in lung inflammation after inhalation of endotoxin or Pseudomonas aeruginosa. Am. J. Physiol **276:**L715-L727.
26. **Staretz-Haham, O., R. Melamed, M. Lifshitz, N. Porat, C. Fieschi, J. L. Casanova, and J. Levy.** 2003. Interleukin-12 receptor beta1 deficiency presenting as recurrent *Salmonella* infection. Clin. Infect. Dis. **37:**137-140.
27. **Sterner-Kock, A., R. K. Braun, M. D. Schrenzel, and D. M. Hyde.** 1996. Recombinant tumour necrosis factor-alpha and platelet-activating factor synergistically increase intercellular adhesion molecule-1 and E-selectin-dependent neutrophil adherence to endothelium in vitro. Immunology **87:**454-460.
28. **Van der Leij, P., H. Amesz, J. Tommassen, and B. Lugtenberg.** 1985. Monoclonal antibodies directed against the cell-surface-exposed part of PhoE pore protein of the *Escherichia coli* K-12 outer membrane. Eur. J. Biochem. **147:**401-407.
29. **Van Diepen, A., J. S. van de Gevel, M. M. Koudijs, F. Ossendorp, H. Beekhuizen, R. Janssen, and J. T. van Dissel.** 2005. Gamma irradiation or $CD4^+$ T cell depletion causes reactivation of latent *Salmonella enterica* serovar Typhimurium infection in C3H/HeN mice. Infect.Immun. **73:**2857-2862.
30. **Ware, C. F., T. L. VanArsdale, P. D. Crowe, and J. L. Browning.** 1995. The ligands and receptors of the lymphotoxin system. Curr. Top. Microbiol. Immunol. **198:**175-218.