

Rapid and Widely Disseminated Acute Phase Protein Response after Experimental Bacterial Infection of Pigs

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Figure 1

Conclusion

The acute phase protein response is a well-described generalized early host response to tissue injury, inflammation and infection, however its biological function(s) and its interplay with other innate host responses are not well-known. It can be considered a humoral innate immune response.

In order to gain new insight into this response in the context of a bacterial infection we studied gene expression changes in peripheral lymphoid tissues as compared to hepatic expression changes 14-18 h after an experimental respiratory infection of pigs. The lung infection was established with the pig specific respiratory pathogen *Actinobacillus pleuropneumoniae*.

The current study demonstrates expression and regulation of acute phase proteins (APPs) during experimental bacterial lung infection in pigs in leukocytes, tracheobronchial lymph nodes, spleen, and tonsillar tissue in addition to the well-known hepatic APP response. The expression changes took place quickly (within 14-18 hours of establishing the infection) and at a scale comparable to the changes in hepatic synthesis and as reflected in serum concentrations of quickly reacting serum proteins serum amyloid A and C reactive protein.

This suggests that many different cell types in the organism are involved in the production of APPs and that extrahepatic APP expression is tightly regulated stressing the biological importance of the APP response.

Materials and Methods

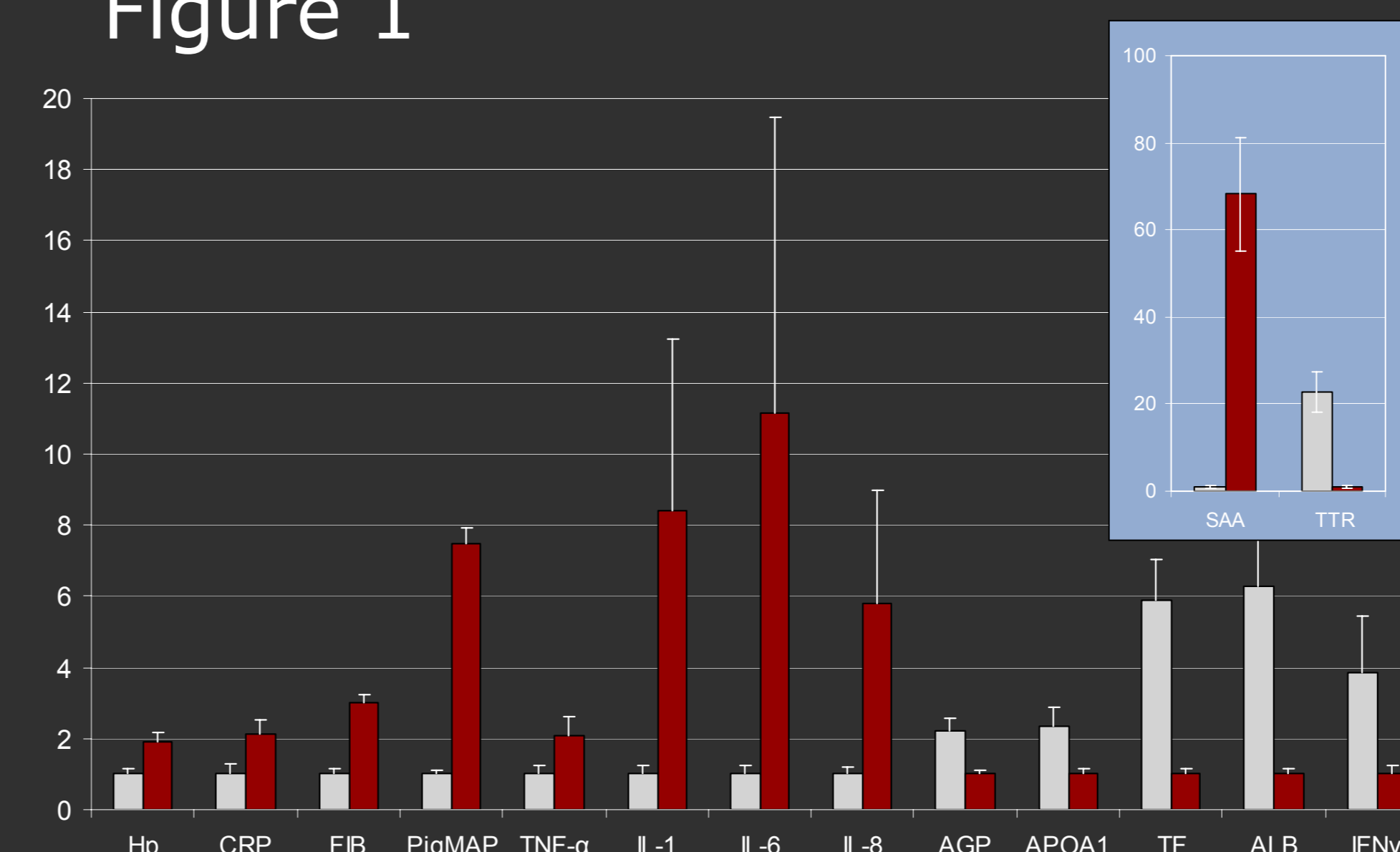
Ten 9-week-old pigs were inoculated with *A. pleuropneumoniae*. Five non-inoculated pigs were used as controls. Animals were sacrificed 14-18h after inoculation. Blood- and tissue samples (liver, tracheobronchial lymph nodes, spleen, tonsils) were taken immediately after sacrifice.

Quantitative real-time RT-PCR (qRT-PCR) was performed to determine differential expression of genes between infected and non-infected animals in blood- and tissue samples. qRT-PCR was performed on a RotorGene 3000 Detection System (Corbett Research, Sydney, Australia). Melting curves were generated after each run to confirm a single PCR product. All reactions were performed in triplicate.

Normalization of gene expression in the tissue of interest was obtained by dividing each sample value by its corresponding normalization factor, based on the geometric mean of the relative concentration of the three best reference genes for this tissue, as described by Skovgaard et al. (2007). The following six reference genes; HPRT1, RPL13A, HTPAP, B2M, GAPDH, and β -actin were ranked based on pair-wise stability using geNorm (Vandesompele et al., 2002).

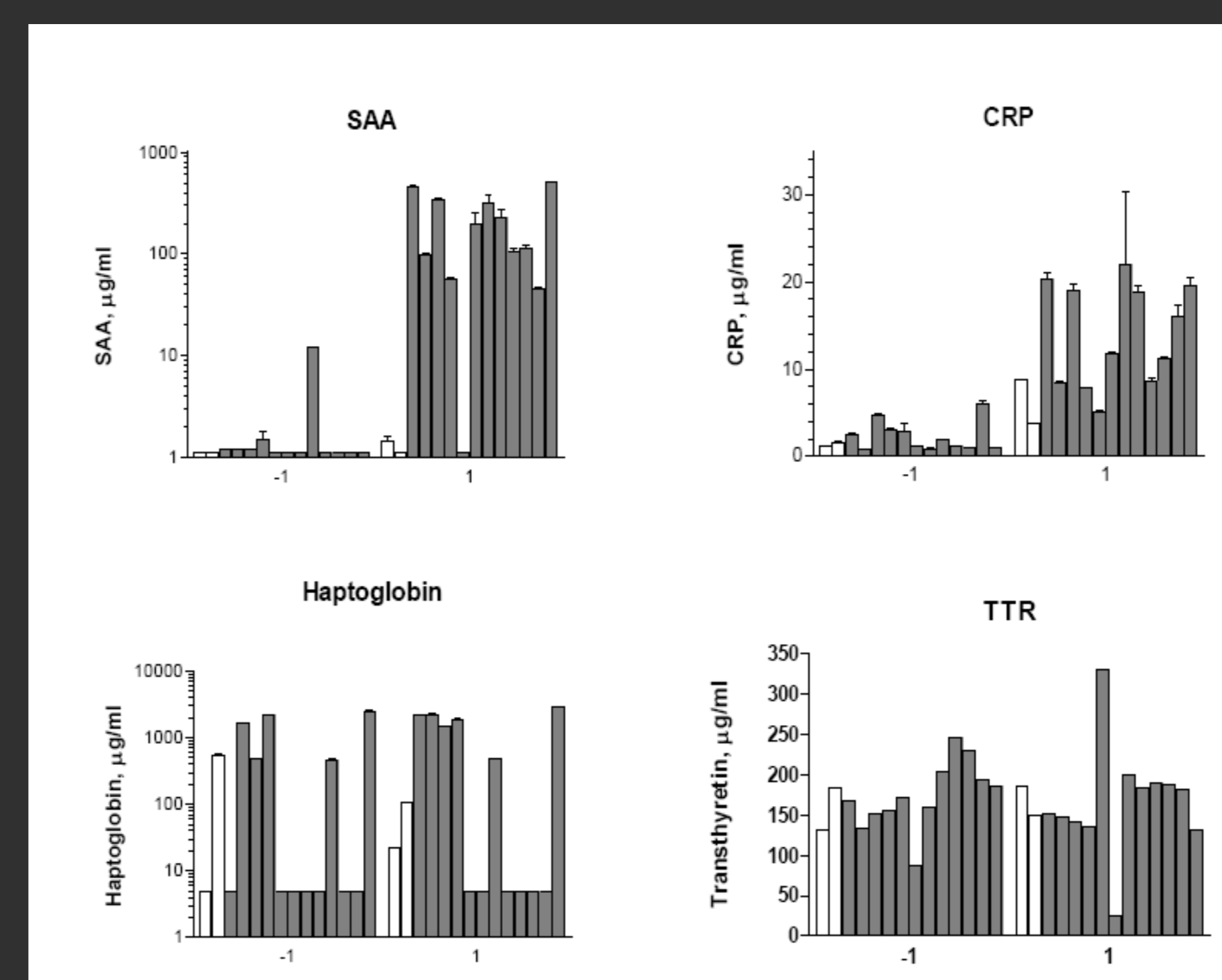
References

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Liver APP expression: mRNA levels normalised and compared to before infection (positive APPs: pre-infection level is set to 1, negative APPs: post-infection level is set to 1)

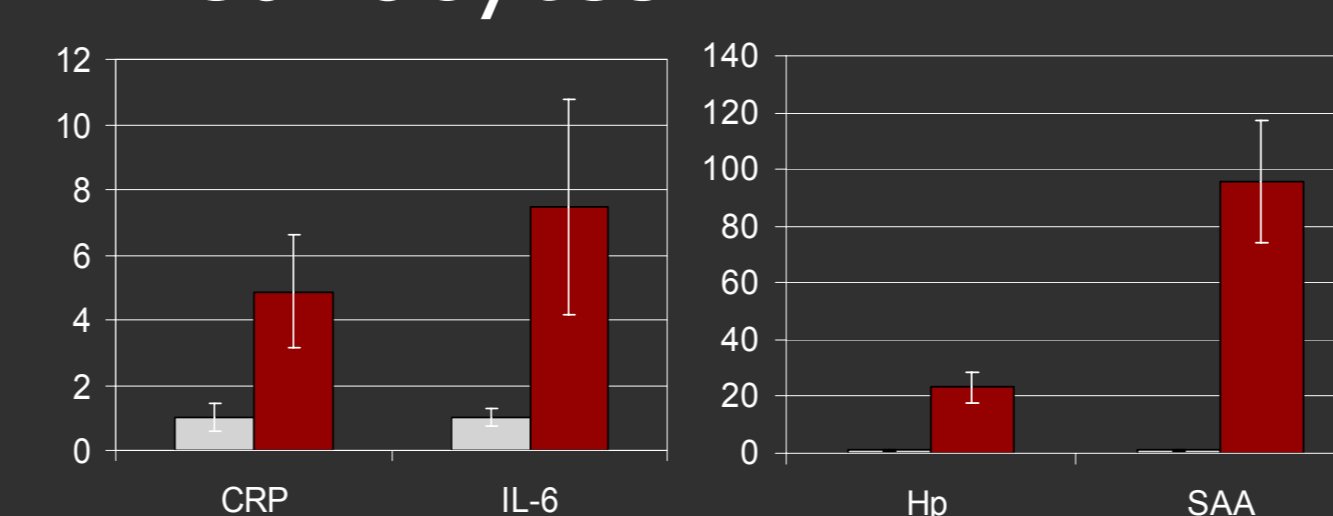
■ Infected (N=10)
□ Control (N=5)



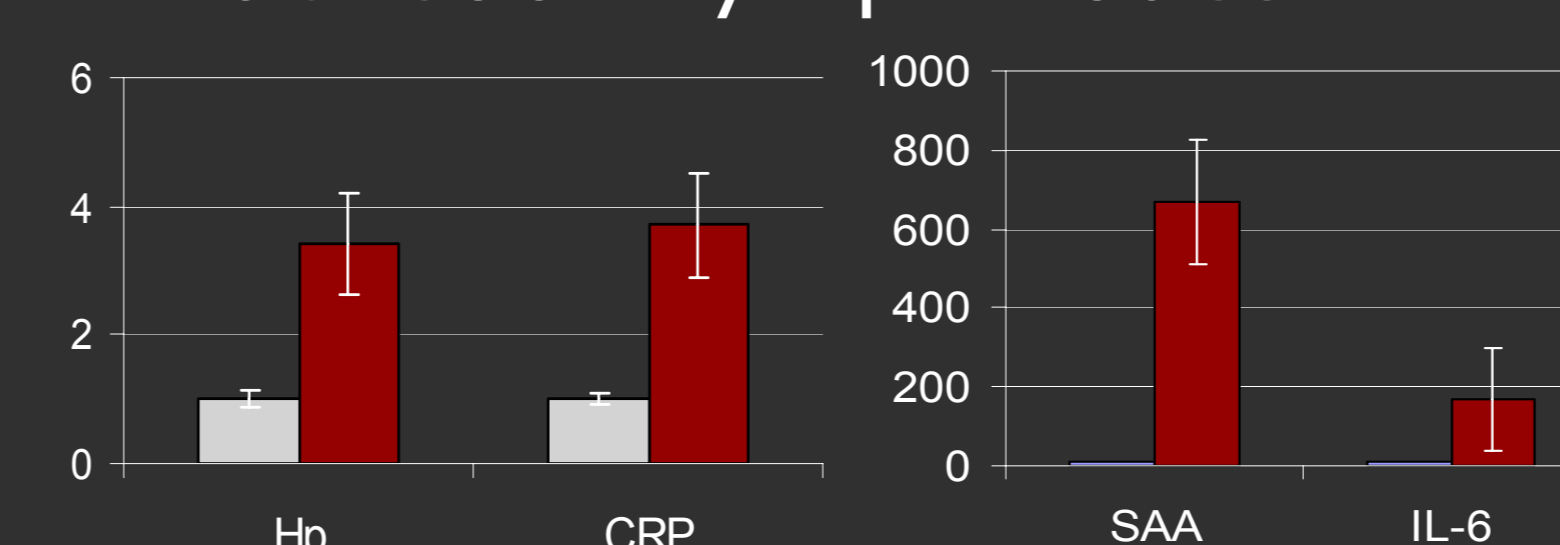
Serum APP response: SAA, CRP, haptoglobin and transthyretin concentrations before and after infection

■ Infected (individual animals)
□ Control (individual animals)

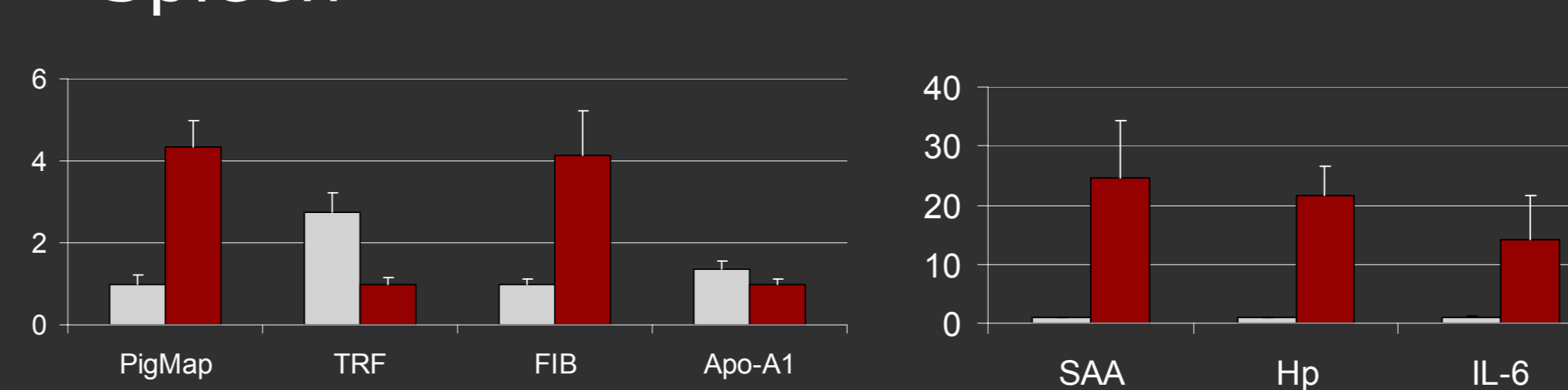
Leukocytes



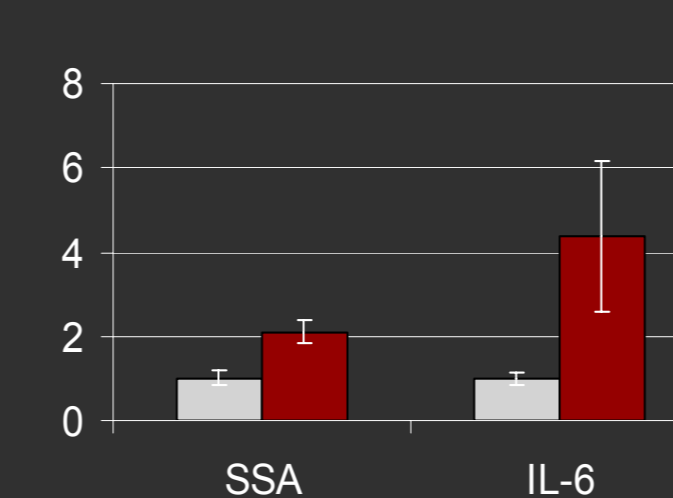
Tracheobr. lymph nodes



Spleen



Tonsil



Non-hepatic APP expression: mRNA levels normalised and compared to before infection (positive APPs: pre-infection level is set to 1, negative APPs: post-infection level is set to 1)

■ Infected (N=10)
□ Control (N=5)

Error bars on all figures: SEM

Results and Discussion

Up-regulated hepatic expression were seen for genes encoding CRP, FIB, HP, PigMAP, SAA, TNF α , IL-1, IL-6, and IL-8 whereas AGP, ALB, APOA1, TTR, TF, and IFN γ were down-regulated (Fig. 1) (also observed previously for some of these genes by microarray analysis by (Hedegaard et al., 2007). The hepatic response was accompanied by expression of ALB, APOA1, CRP, HP, PigMAP, SAA, TF, TTR, and IL-6 in tonsillar, splenic, and tracheobronchial lymphatic tissue as well as in leukocytes (Fig. 1).

This Extrahepatic APP expression is most probably a reflection of specific local tissue cell expression, as several APPs were expressed at a significantly different level in the various tissues compared to leukocytes as exemplified with SAA in Fig. 2.

Thus regulation of APP expression is not confined to the liver but is widely disseminated and involves a large number of APPs throughout the body at a dynamic scale comparable to the hepatic response.

It would be very interesting to know more about the exact cell types involved and to elucidate the nature of the stimulus causing this disseminated cytokine (IL-6) and APP response.

Figure 2

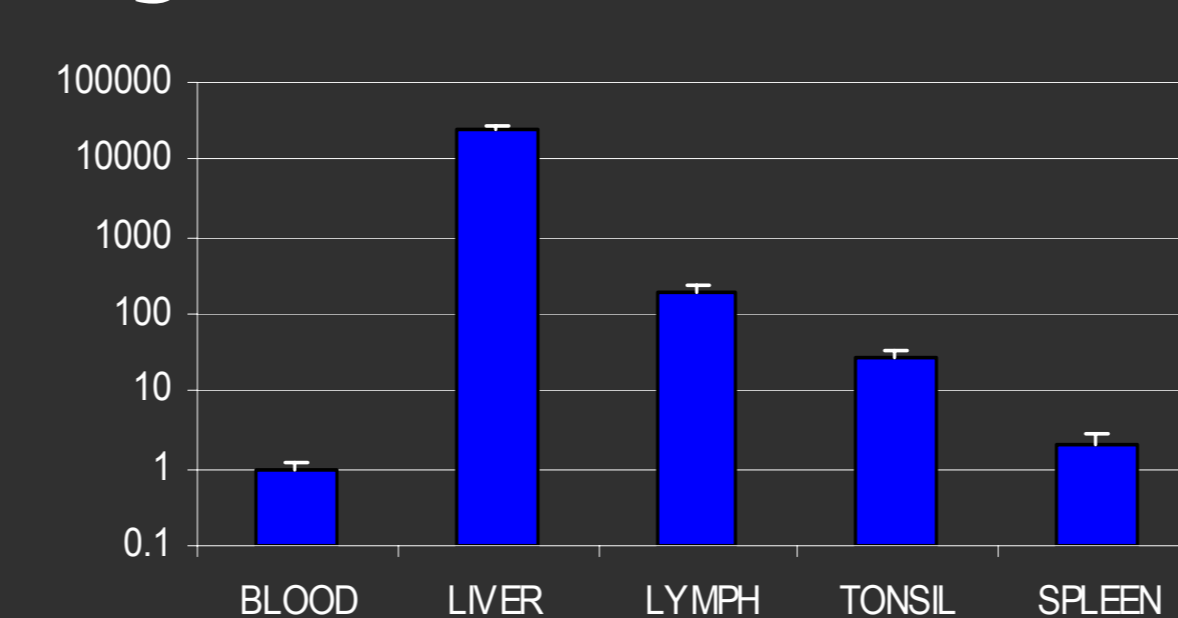


FIGURE 2. Serum amyloid A2 expression changes in different tissues in the group of infected animals (N=10). Blood leukocyte expression values were set to 1. Standard error of the mean (SEM) is illustrated by error bars.