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IMMUNOBLOT ANALYSIS OF IgG SUBCLASS ANTIBODY RESPONSE AGAINST *COXIELLA BURNETII* IN BALB/C MICE

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Summary. - Balb/c mice were inoculated with live or inactivated organisms of *Coxiella burnetii,* strain Nine Mile, phase I. Sera collected after different time intervals were subjected to immunoblot analysis and results compared with ELISA values. Immunoblots were performed with different horseradish peroxidase labelled conjugates (goat anti-mouse IgG_1, IgG_{2a}, IgG_{2b}, IgG_3) and results monitored and analysed by laser densitometry. ELISA analysis was performed using the same peroxidase labelled goat anti-mouse subclass antibody conjugates. In addition, goat anti-mouse IgG(H+L), IgG(H), IgM, and IgA conjugates were used for ELISA tests.

Key words: Coxiella; immunoglobulins; immunoblot; laser densitometry; quantification; experimental ingection

Serology for the detection of Q fever infection with *Coxiella (C.) burnetii* today is mainly based on immunofluorescence (IF), the complement fixation test (CF) and on enzyme-linked immunosorbent assays (ELISA). Results of these tests provide the scientist or clinician with useful, however restricted information. The CF test or the ELISA are like „black boxes". To decide upon acute or chronic infection at least IFT and ELISA are able to classify an immune response more comprehensively when using class- or subclass-specific conjugates for the differentiation of specific IgM, IgA, or IgG. In order to get a broader understanding of the immune response of *C. burnetii* infected humans or animals an immunoblot system for detection and classification of the specific antibody response was developed and is described in this communication. Mice were used as a model and special emphasis was placed on IgG subclass responses. Since visual analysis of immunoblot results does not allow quantification of specific immune responses, laser densitometry was applied for this purpose.

Balb/c mice were inoculated with live or inactivated organisms of *C. burnetii,* Nine Mile, phase I propagated in embryonated hen's eggs in contrast to antigen

used in the different test procedures which was propagated in cell cultures. Sera collected after different time intervals were subjected to immunoblot analysis and results compared with ELISA values.

Since SDS-PAGE with formalin-inactivated antigen failed to produce appropriate results, we used native Coxiellae instead. This led to more than 50 distinct antigen bands after Western blotting. These experiments were performed with Immobilon membranes (Millipore) instead of nitrocellulose.

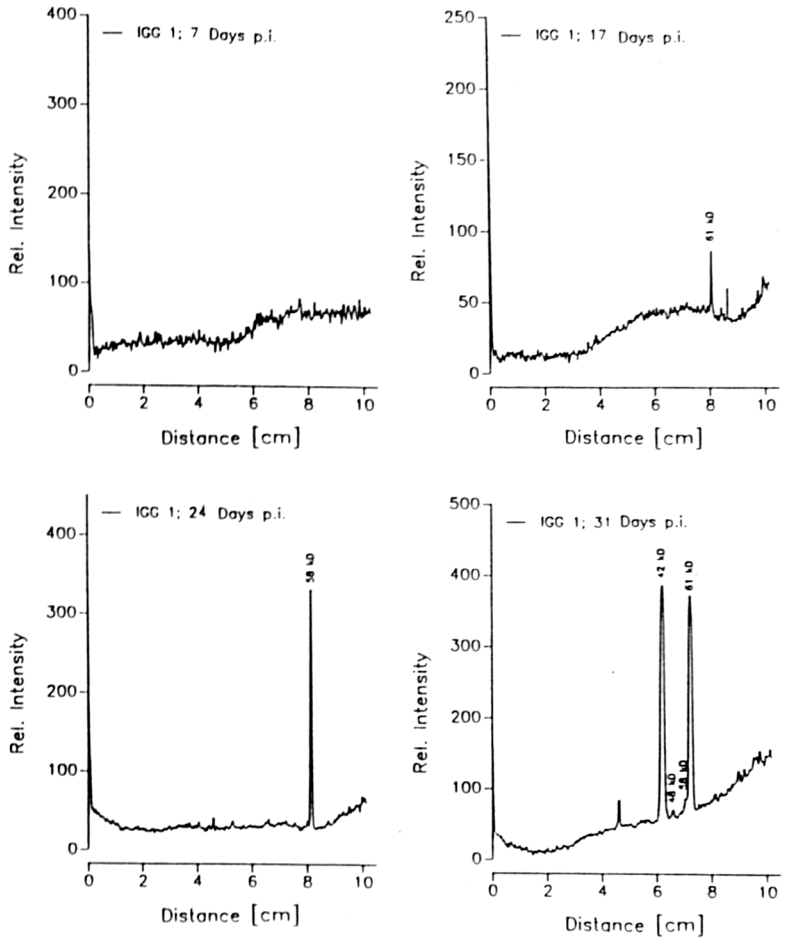

Fig. 1
Immunoblot data obtained from 1 infected mouse using antimouse IgG1 antibodies after different time intervals

In the meantime, a membrane with higher protein binding capacity - from PALL (Fluorotrans) - proved to be even superior for this purpose. To reduce background fish gelatine was used for the immunoblot test procedure to saturate free binding sites. To further increase specificity and sensitivity glutaraldehyde fixation described by Ikegaki (1989) was carried out after the antibody incubation step. Immunoblots were performed with different horseradish peroxidase-labelled conjugates [goat anti-mouse IgG(H-chain), IgG1, IgG2a,

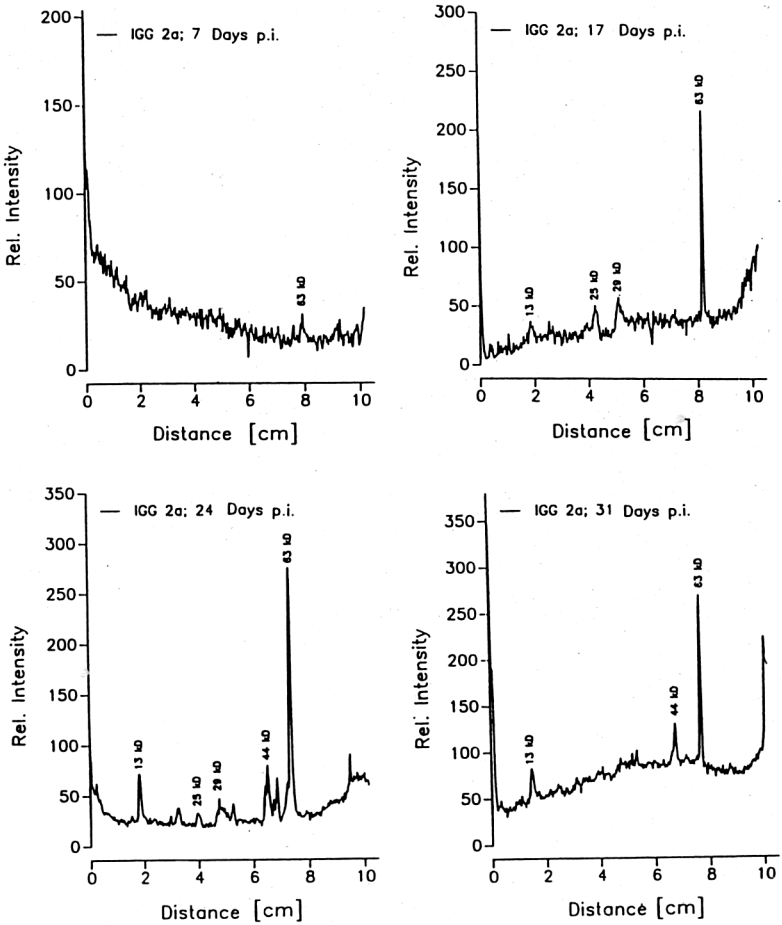

Fig. 2
Immunoblot data obtained from 1 infected mouse using antimouse IgG2a antibodies after different time intervals

IgG2b, IgG3, and for comparative reasons IgM and IgA]. For comparison and quantification of the results a standard positive mouse serum was used.

For quantification immunoblots were read not only visually but be means of a LKB laser densitometer. Immunoblot stripes were photographed immediately after development, positives produced in the usual way and the photographs directly subjected to laser densitometry. Accurate and reproducable quantification of stained bands was obtained. After reading a strip the instrument supplied data which were transmitted to an IBM personal computer and each stained band on the strip was monitored as a peak. Results were expressed as integrals representative of the area under the curve drawn. The area under a peak was proportional to both stain intensity and the width of the band on the

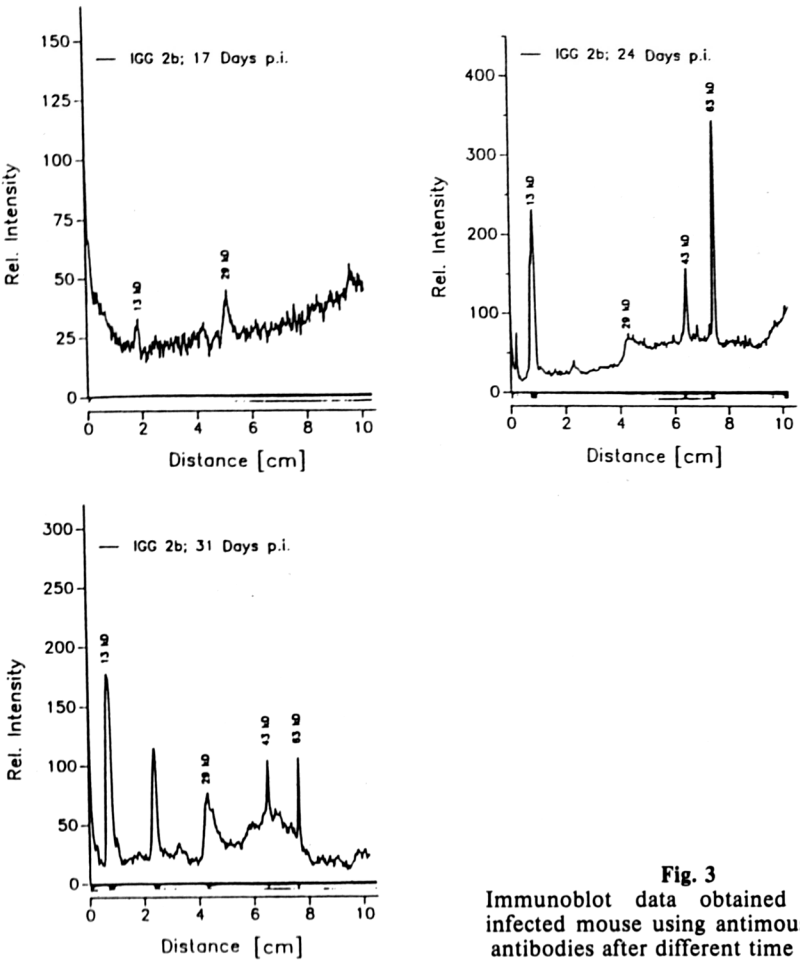

Fig. 3
Immunoblot data obtained from 1 infected mouse using antimouse IgG2b antibodies after different time intervals

strip. The areas integrated were comparable between series of readings by means of calibration against a positive reference serum using a computer program developed by one of us (H.W.).

Bands of standard molecular weight markers for calibration were not stained by chemical staining; to reduce length differences of membranes resulting from different staining procedures they were developed immunologically using specific rabbit antiserum against the marker proteins. For ELISA analysis the same peroxidase labelled goat anti-mouse class- and subclass-antibody conjugates were used. Conjugates were extensively tested for cross reactivity using different class and subclass monoclonal antibodies as reference. To get comparable quantitative results a dilution factor for each conjugate was

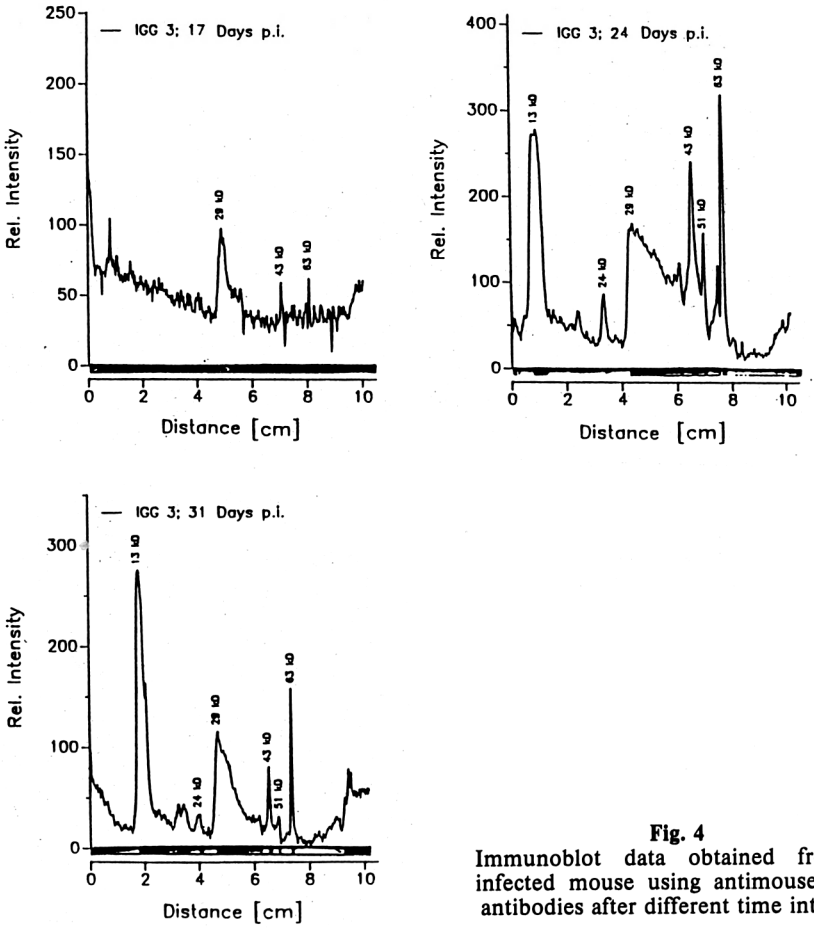

Fig. 4
Immunoblot data obtained from 1 infected mouse using antimouse IgG3 antibodies after different time intervals

selected after chequerboard ELISA to yield the same ELISA value with a certain monoclonal antibody concentration.

The immune response detected by immunoblot for IgG1 antibodies differed in intensity but was rather homogeneous concerning the recognition of single proteins bands. Sera of all nine mice under investigation recognized bands with a molecular weight of 42, 48, 58, and 63 kD showing a predominant reaction with the 63 kD band. The immune response for IgG2a was homogeneous in intensity. Protein bands recognized by sera from all mice had molecular weights of 13, 25, 29, 44, and 63 kD. An intesive reaction was detected with the 63 kD band. The immune response for IgG2b and IgG3 was rather similar for all nine infected mice. Proteins revealed by all mice with IgG2b antibodies were of 13, 43, and 63 kD MW whereas IgG3 antibodies were bound especially by proteins with a MW of 13, 29, 43, and 63 kD. A strong reaction was detected with antigens of 13, 29, and 63 kD. These results demonstrate that there were considerable differences in the immune response with regard to IgG subclasses.

As an example, Fig. 1 to 4 demonstrate the immunoblot data obtained from infected mouse after different time intervals revealed by laser densitometry and computer analysis.

Although immunoblot investigation is a very sensitive and sophisticated tool for analysis in serology, comparison of different immunoblots by visual recognition of bands is a rough and not very objective method. With the aid of laser densitometry immunoblots may be scanned and submitted to computer analysis thus resulting in more reliable quantitative and qualitative results that can be easily compared. Such results should also lead to a more accurate understanding of the immune response in view of vaccine production or, for example, production of monoclonal antibodies.

References

Ikegaki, N., and Kennett, R. H. (1989): Glutaraldehyde fixation of the primary antibody-antigen complex on nitrocellulose paper increases the overall sensitivity of immunoblot assay. *J. immun. Meth.* **124**, 205–210.