Acta virol. **36**: 52-56, 1992

PROTEIN ANTIGENS OF GENETICALLY RELATED *RICKETTSIA PROWAZEKII* STRAINS WITH DIFFERENT VIRULENCE

N. M. BALAYEVA, M. E. EREMEEVA, V. F. IGNATOVICH, B. A. DMITRIEV, E. B. LAPINA, L. S. BELOUSOVA

The Gamaleya Institute of Epidemiology and Microbiology, Academy of Medical Sciences U.S.S.R., 123098 Moscow, U.S.S.R.

Received March 21, 1990

Summary. - The protein antigens of two distinct lines of genetically related strains, namely the nonpathogenic strain E and its virulent revertant EVir and of the standard virulent strain Breinl were compared in SDS-PAGE and immunoblot assay using typhus patient sera and immune rabbit sera. No differences in the polypeptide pattern as detected in SDS-PAGE were found between strain E and EVir; the Breinl strain differed in a 30 kD protein. The high immunogenicity of the protein antigens of E, EVir and Breinl strains was demonstrated by immunoblot assay with human sera, which did not show any differences between the strains studied. Immunoblot analysis with immune rabbit sera to the strain E, EVir, and Breinl showed differences in immunological response to the 70 kD and 60 kD polypeptides of low virulent strain E and those of virulent strains EVir and Breinl.

Key words: Rickettsia prowazekii; weakly pathogenic strain E; virulent revertant strain EVir; protein antigens

Introduction

At present one pair of genetically related strains of *Rickettsia prowazekii* with different virulence is known: E and EVir. Strain E is unique among strains of *R. prowazekii* because of its low virulence for man and some experimental animals (Clavero and Perez-Gallardo, 1944). However, avirulent properties of strain E are not stable. Passaging of strain E under certain experimental conditions resulted in a stable enhancement of the strain virulence. This reversible strain was marked as strain EVir of *R. prowazekii* (Balayeva and Nikolskaya, 1973). Genetic relations of E and EVir strains were confirmed by restriction DNA analysis (Balayeva *et al.*, 1989). The basis of strain E attenuation and mechanism of enhancement of its virulence is unknown.

This paper presents the characteristics of protein antigens of two independent lines of strains E and EVir and virulent strain Breinl of *R. prowazekii.*

Protein antigens of the strains studied were compared by SDS-PAGE and immunoblot assay with the use of immune human and rabbit sera to *R. prowazekii.*

Materials and Methods

R. prowazekii. Two distinct lines of genetically related strains E 281 - EVir 281 and E 20 - EVir 20 (Balayeva and Nikolskaya, 1973) and virulent strain Breinl passaged in chick embryos were studied. Strains E 281 and E 20 - passage lines of strain E received from U.S.A. in 1955 and 1969, respectively. Virulent strains Evir 281 and EVir 20 were obtained by intranasal inoculation in white mice: strains E 281 and E 20 during 12 and 13 passages, respectively, then 4 and 10 passages in chick embryos, respectively.

The procedure of the purification of rickettsiae by Verografin (SPOFA, C.S.F.R.) density gradient centrifugation, the methods of preparation of immune rabbit sera against each strain of *R. prowazekii* were described by Aniskowich *et al.* (1989). SDS-PAGE was performed according to Laemmli (1970), immunoblot assay with the use of typhus patient's sera and immunized rabbit antisera to strains E, EVir, and Breinl of *R. prowazekii* was performed as described by Eremeeva *et al.* (1989).

Results and Discussion

SDS-PAGE of the whole cell lysate of purified *R. prowazekii* strains E, EVir, and Breinl revealed as many as 55 polypeptide bands including the major bands corresponding to protein M_r 130, 60, 32, 25, and 17 kD (Fig. 1), marked in conformity with Eisemann and Osterman's classification (1976) as I-VI, respectively. This analysis has not revealed any differences in polypeptide distribution between two independent lines of strains E and EVir. The results obtained testify that polypeptide composition of strain E and EVir is stable and characteristic for these strains. The polypeptide distribution of E and EVir strains was similar to that of virulent Breinl strain except of a distinct protein in the 30 kD (IV) position. The latter major protein of strain Breinl shows lower electrophoretic mobility in comparison with the E and EVir strains.

The distinction in the protein IV position between avirulent strain E and virulent strain Breinl was shown by Oaks *et al.* (1981). These authors suggested a relationship between protein IV and the virulence of *R. prowazekii.* However, the identity of the polypeptide composition of the genetically related strains E and EVir of different virulence does not confirm this suggestion. Nevertheless, it should not be exluded that the increased electrophoretic mobility of protein IV in strain E is a phenotypical expression of strain E attenuation and the reversion of its virulence is due to another strain E modification. It should be noted that the distinction in protein IV position has not been found yet among other *R. prowazekii* strains studied (Dasch *et al.,* 1978; Oaks *et al.,* 1981). The protein analysis of the virulent strain Madrid E I, the parent strain of strain E (Clavero and Perez-Gallardo, 1944) as well as of other *R. prowazekii* strains would be of

value for the study of the relationship of protein IV with the virulence or the strain feature of *R. prowazekii.*

The results of the study of antigenic properties of polypeptides derived from strains E, EVir, and Breinl by immunoblotting with the use of typhus patient sera and immune, rabbit antisera to three strains are summarized in Figs. 2–7. The typhus patient sera demonstrate the high immunogenicity of the protein antigens of strains E, EVir, and Breinl including minor and major proteins, without any differences between the strains studied. The major protein I M_r 100–130 kD was recognized as a main antigen. The immunogenicity of protein antigens of the given strains was lower with rabbit sera. The latter used in immunoblot assay did not show any differences between the strains studied, as well.

Immunoblot assay with rabbit antisera to strains E, EVir, and Breinl showed that the sera to virulent strains discriminated the strain E, detecting a different protein pattern and by a different dynamics of antibody synthesis. On the 30th day after infection with strain E anti E-serum did not detect the major protein M_r 60 kD in any of the strains compared, however, it precipitated intensively the minor protein M_r 70 kD. In contrast, the rabbit serum raised against the virulent strains EVir or Breinl drawn on 30th day post-infection detected the major protein M_r 60 kD but not the minor protein M_r 70 kD. At very late intervals (300th day post-infection) no differences in the ability of sera to detect the major protein M_r 60 kD and the minor protein M_r 70 kD were found.

Summing up, it was established that the genetically related strains E and EVir have the same protein composition and are distinguishable from the viru-

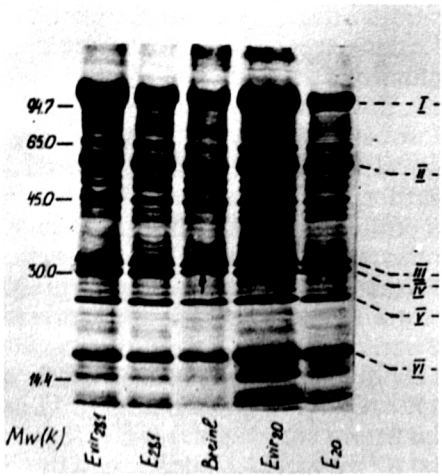
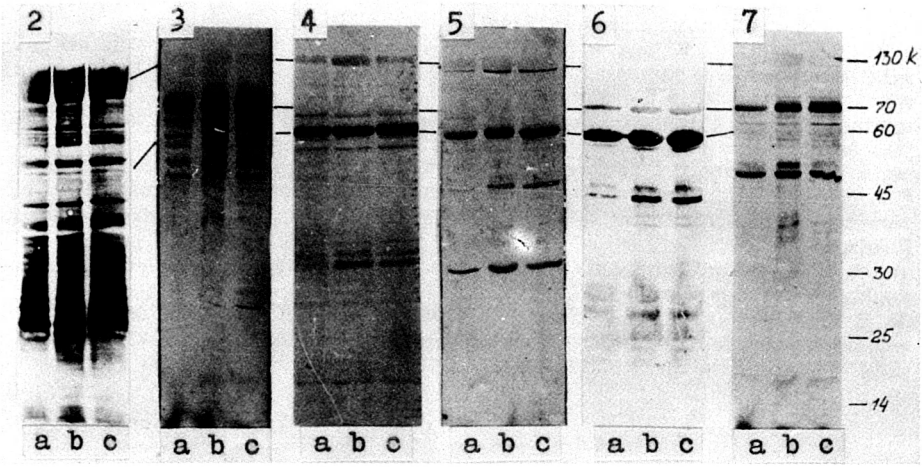


Fig. 1
Protein analysis of *Rickettsia prowazekii* strains E, EVir, and Breinl in 7.5 - 15 % SDS-polyacrylamide slab gel
Purified rickettsiae were solubilized in the buffer containing 2 % SDS and 5 % 2-mercaptoethanol heated in a boiling water bath for 5 min and applied to each lane (25 μg protein): strains EVir 281 and EVir 20 were obtained by passaging in lungs of white mice of strain E, passage lines 281 and 20, respectively; low virulent strains E 281 and E 20 passaged in chick embryos were received from the U.S.A. in 1955 and 1969, respectively. I-VI indicate the major proteins of rickettsiae. Arrow indicates the protein IV of strain Breinl with distinct electrophoretic mobility from strains E and EVir. Molecular weight standards (Pharmacia) in the left (MwK).



Figs. 2–7
Immunoblotting profiles of *R. prowazekii* strains E, EVir, and Breinl
Fig. 2. Serum from a typhus patient (1:2000).
Fig. 3–5. Rabbit sera on the 30th day post-infection with strain E (1:1 500), EVir (1:3 000), Breinl (1:2 000) respectively.
Figs. 6–7. Rabbit sera on the 300th day post-infection with strains E (1:400), and EVir (1:400), respectively.
R. prowazekii strains: a – Breinl, b – EVir, c – E. Molecular weight protein standards (Pharmacia) in the right.

lent strain Breinl by the electrophoretic mobility of the major protein M_r 30 kD only. Strain E showing low virulence and the virulent strains EVir and Breinl were found to exhibit identical proteins when reacting with typhus patient sera and rabbit immune sera. Immunoblot analysis with rabbit antisera to strains E, EVir, and Breinl showed differences in the dynamics of antibody response to polypeptides M_r 70 kD and 60 kD between the low virulent strain E on one hand and the virulent strains EVir and Breinl on the other hand.

References

Aniskovich, L. P., Eremeeva, M. E., Balayeva, N. M., Ignatovich, V. F., Artemiev, M. I., Emelyanov, V. V., and Smirnova, N. S. (1989): Methods for purification of *Rickettsia prowazekii* separated from the host tissue: a step-by-step comparison. *Acta virol.* **35**, 361–370.

Balayeva, N. M., and Nikolskaya, V. N. (1973): Increased virulence of the E vaccine strain of *Rickettsia prowazekii* passaged in the lungs of white mice and guinea pigs. *J. Hyg. Epidem. Microbiol. Immunol.* **17**, 11–20.

Balayeva, N. M., Rydkina, E. B., Artemiev, M. I., and Ignatovich, V. F. (1989): Restriction endonuclease analysis of the DNA of *Rickettsia prowazekii* vaccine strain E and its revertant. *Acta virol.* **33**, 454–464.

Clavero, C. G., and Perez-Gallardo, P.F. (1944): Experimental study of a nonpathogennic immunizing strain of *Rickettsia prowazekii. Trop. Dis. Bull.* **4**, 24.

Dasch, G. A., Samms, J.R., and Weiss, E. (1978): Biochemical characteristics of typhus group rickettsiae with special attention to the *Rickettsia prowazekii* strains isolated from flying squirrels. *Infect. Immun.* **19**, 676-685.

Eisemann, C. S., and Osterman, J. V. (1976): Proteins of typhus and spotted fever group rickettsiae. *Infect. Immun.* **14**, 155-162.

Eremeeva, M.E., Lapina, E. E., Balayeva, N. M., Ignatovich, V. F., Belousova, L.S., and Dmitriev, B. A. (1989): Electrophoretic and immunochemical characterization of proteins from *Rickettsia prowazekii* strains differing in virulence. *Molek. gen. Microbiol. Virusol.* **1989 (5)**, 20-26 (in Russian).

Laemmli, U. K. (1970): Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature (London)* **227**, 680-685.

Oaks, E. V., Wisseman, C. L., and Smith, J.F. (1981): Radiolabelled polypeptides of *Rickettsia prowazekii* grown in microcarrier cell cultures, pp. 461-472. In W. Burdorfer, R. L. Anacker (Eds): *Rickettsiae and Rickettsial Diseases,* Academic Press, Inc., New York.