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IMMUNOLOGICAL DIAGNOSIS IN VIVO AND ACAPSULAR ANTHRAX VACCINE: A PIONEER ACHIEVEMENT OF ROMANIAN MEDICAL SCIENCE

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Abstract: This review presents many of numerous studies realized in the past century by Romanian researchers who contributed directly or indirectly to progress in animal anthrax specific prevention and imunological diagnosis of the disease. It is underlined Nicolae Stamatin's great contribution; he obtained a new acapsulated and attenuated vaccinal strain of *B. anthracis* whose mass use led to a dramatical decrease of anthrax morbidity among animals.

Many studies were performed to achieve an immunological diagnosis of the disease. The review highlights the studies performed by Shlyakhov et al. on laboratory diagnosis of patients which are culture negative because of previous antibacterial therapy. The intradermal testing with Anthraxin extracted from acapsulated vaccinal strain of *B. anthracis* by Shlyakhov et al. offers the possibility to asses the cell mediated immunity. Anthraxin-like products adapted for ELISA will be able to reveal, *in vitro*, the cellular immunity in vaccinated animals.

Keywords: anthrax, vaccine, immunological diagnosis

Rezumat: Lucrarea preyintă contribuția specialiştilor români, în ultimul secol, la progresele în domeniul prevenirii antraxului animal şi la diagnosticul imunologic al bolii. Este subliniat meritul prof. N. Stamatin de a reuşi obținerea unei tulpini vaccinale acapsulată şi atenuată de *B. anthracis* prin a cărei utiliyare s-a redus considerabil morbiditatea prin antrax la animale. Sunt prezentate, de asemenea, rezultatele cercetărilor efectuate de colectivul prof. E. Shlyakhov cu preparatul Antraxin extras din o tulpină vaccinală acapsulată, răspunsul imun mediat celular, posibilitatea de adaptare pentru ELISA.

Cuvinte cheie: antrax, vaccin, diagnostic imunologic

In the first third of the twentieth century anthrax among farm animals and humans remains still an actual problem affecting human health and national economy of concerned countries, including Romania - which at that time was mainly an agricultural land.

In the past century the Romanian school of veterinary and medicine contributed to the study of bacteriology, pathogenesis, epizootology and epidemiology of this infection.

The most important contribution was in the specific prophylaxis of the disease.

It is worth mentioning Cernaianu and Suhateanu`s studies (1924, 1932) on tissue receptivity in anthrax experimental infection (1,2).

These studies were continued by Cornelson, Toma and Constantinescu, demonstrating the cerebral sensitivity of "refractory" animals to *B. anthracis* (1949) (3).

This way, the hypothesis proposed by Besredka on the exclusive coetaneous receptivity was infirmed.

Studies by Combiescu, Şoru et al. (1929) (4) on *B. anthracis* specific solubles substances, those published by Ionescu-Mihăieşti (5) on bacillus antigenic structures (1937) and the study by Ciucă M. (1950) (6) on the susceptibility to bacteriofage of the local isolates of *B. anthracis* represented at that time a pioneer achievement.

One of the most important target in the list of anti-epizootic measures which kept urgent attention was the necessity of an improved anthrax vaccine. Thus, the world wide employed since 1885 the two Pasteur's vaccines -I and II - were not deprived of major deficiencies: they were poorly standardized some series demonstrating a significant residual virulence or, in a opposite side, being inactive, and generally, they showed lack of safety (7). To overcome these deficiences Nicolae Stamatin, an outstanding Romanian veterinary microbiologist obtained in 1936 a new not-capsulating and attenuated vaccinal strain of *B.anthracis* called 1190R. This happened **two years before** that a similar strain, called 34 F2 was performed in 1938 by Max Sterne in Onderstepoort, South Africa (8,9).

Their performance was followed by Nicolae Ginsburg and Alexander Tamarin (USSR) who obtained a similar vaccinal strain called STI, firstly intented for use in veterinary practice and later, to serve as a prototype strain for a human live anthrax vaccine (10). Stamatin's vaccine is succesfuly employed up to present in Romania, while the both kinds of the STI vaccine are used in Russia and CIS countries, and the Sterne vaccine serves until now for world-wide animal immunization. All the non-capsulating anthrax vaccines are generally well tolerated. Owing their enhanced immunogenicity and low residual virulence their mass use led to a dramatic decrease of anthrax morbidity among animals, and, subsequently, brought down the incidence of anthrax in men.

Additionally, the mass introduction since 1940 of antibiotics reduced the rate of lethal exits both in men and animals.

Unfortunately, the wide use of antibiotics led to a notifiable decrease of the rate of bacteriological confirmation in acute patients as a result of early dead of the pathogen under treatment. Thus, if before the large use of antibiotics (1920-1945) the averaged isolation rate of the pathogen varried from 52.2% to 84%, after the introduction of antibiotics the pathogen was isolated in less than 30% of acute anthrax cases (11). At that time, no any alternative either microbiological neither immuno-logical method was available and a lot of conventional, having a good rating in other infectious diseases, serologic methods e.g. the reaction of agglutination, the

complement fixation, and their varieties were tryied for anthrax without success.

Search for a simple and reliable test for assessment post-infectious and post-vaccinal immunity in anthrax was additionally stressed by the fact that excepting a virulent challenge no other possibility to evaluate this status in animals existed at this time. Moreover, it goes without saying that the challenge method is absolutely impracticable in humans.

Meanwhile, some separate publications since 1924 indicated that the pathogen can induce *in vivo* a hypersensitive status in susceptible animals. Thus, Zironi obtained a delayed "skin allergy" when introduced intradermally in vaccinated against anthrax guinea pigs live or inactivated by formalin *B.anthracis* organisms (12). Later, in 1927 Hruska in a vaccination assay on horses injected simultaneously with live virulent B. anthracis organisms and sterile edema fluid collected from vaccinated guinea pigs. Following a repetead subcutaneous injection of small amounts of sterile edema fluid taken from immunized guinea pigs into vaccinated horses a large local inflammation was observed (13). Many years later, in 1943, Romanian researchers Balteanu, Toma and Garaguli published a paper entitled: "Principe oedematogene et allergie charbonneuse" (14) which descripted their assay with sterile edema fluid collected from rabbits injected with a virulent *B.anthracis* culture.

This product when injected intradermally 0.1 - 0.2 ml in patients suffering from acute anthrax or who suffered in the past provoqued large local inflammatory reactions (until 12 cm in size or more) in all exposed individuals tested. Unfortunately, about 35% control healthy patients gave similar inflammatory reactions and in some cases local skin necroses were observed. That is why, this method was temporary abandoned or was used exceptionally.

One contribution to the rapid diagnosis of human infection was the adaptation of Ascoli reaction by Cornelson, Toma, Vâță et al. (1951) (15) who used oedema and vesicular liquid in the precipitation test.

Further improvement of the skin test for anthrax was based on the new approaches of the technology of preparation of the edema fluid in the aim to eliminate non-specific and tissue-damaging reactions. Following a close analysis of the antigenic structure of the crude edema fluid Shlyakhov suggests that the tissue damaging substances are mainly linked with the capsular material of the virulent pathogen which diffuses into the edema during the infectious process. This suggestion proved its value. Indeed, when instead of virulent pathogen, one of attenuated and non-capsulating but fully edematogenous vaccinal strain (STI, 34 F2 or 1190 R) was used, the author obtained within 1957 and 1962 some preparations of a more specific and absolutely safe diagnostic product.

Finally, the specific diagnostic material was extracted **directly** from the non-capsulating vaccinal strain of *B.anthracis* (either STI or 34 F2) using a mild chemical procedure. This product was called "Anthraxin" and being commercialized in USSR since 1962 until present.

In contrast with previous serologic methods Anthraxin was based on the reveal of anthrax cell-mediated immunity induced either by the pathogen or by the live vaccine in susceptible animals (guinea pigs, sheep, horses, monkeys, hamsters a.o.), but also in humans suffering from anthrax in present or in the past (until 35 years ago). (For details see references 16,17,18). It is appropriate to make mention here only of some principal results of the evaluation of the anthraxin skin test for diagnosis of acute and past human anthrax (19). Thus, the test was positive in 81.8% of cases in the first three days of the acute disease, and in 97-99% of cases in the next two to three weeks.

The positivity rate was 98.5% in the first 1.5 months of convalescence, 92.8 in the next 3 years, 82.8% in the following 4 to 15 years, and 72.7% 16 to 31 years after recovery. In contrast the bacteriogical confirmation on the same lot of acute patients investigated oscillated between 41.7 and 32% in the first week of disease, was 18.1% in the second week and 10.6 to 0% in the following three weeks after onset of the disease.

The above results were obtained in skin testing of 984 patients with acute cutaneous anthrax, and 950 convalescents. To day anthraxin is recommended for diagnostic purposes by the WHO (19). Recently, in 1999 Stepanov et al.(20) stressed that the anthraxin skin test was the unique method to validate the human live vaccine STI in the former USSR, allowing for the first time to evaluate the optimal doses for cutaneous, subcutaneous and aerosol ways of vaccination. Some unpublished russian data testify that even at present about 80% of all diagnoses of human anthrax are confirmed using anthraxin.

In the last year Shlyakhov and co-workers demonstrated in laboratory experiments that a purified anthraxin-like product was able to reveal *in vitro* (using ELISA, and also the test of stimulation of sensitized lymphocytes or splenocytes) the anthrax cell-mediated immunity in vaccinated animals. These findings open a new and perspective way of ongoing research in this field.

There are, briefly, the milestones and the ongoing steps in a further development of a pioneer achievement of the Romanian medical science which allowed to obtain an improved anthrax live vaccine both for veterinary and human (in Russia) use and in the same time to perform a new technology of a diagnostic product "Anthraxin" suitable for *in vivo* and *in vitro* investigations of anthrax cell-mediated immunity.

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