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THE HUMORAL IMMUNE RESPONSES OF PATIENTS BITTEN BY THE SNAKE *BOTHROPS JARARACA* (*JARARACA*)

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M. O. DOMINGOS, J. L. CARDOSO, A. M. MOURA DA SILVA and I. MOTA. The humoral immune responses of patients bitten by the snake *Bothrops jararaca* (*jararaca*). *Toxicon* **28**, 723–726, 1990.—The isotype and specificity of antibodies produced by patients bitten by *B. jararaca* and submitted to serum therapy were studied. The IgG anti-*B. jararaca* antibodies have large individual dispersion, starting to appear 10 days after the first bite and increasing to at least 80 days after the bite. IgM antibodies appeared sooner than IgG antibodies but disappeared about 20 days after the bite. Secondary responses induced by an additional bite were characterized by a fast and higher IgG antibody response with no apparent change in the IgM antibody. The immunoblotting tests showed that the specificity of human anti-*B. jararaca* antibodies is heterogeneous, each patient recognizing different fractions in the *B. jararaca* venom.

VERY LITTLE is known on the isotype and specificity of antibodies produced by patients bitten by poisonous snakes. We report here the results of a study on the anti-*Bothrops jararaca* venom antibody production, its isotype and specificity using the ELISA and western blotting techniques. The study was performed with patients bitten by *B. jararaca* with envenomation confirmed by clinical and haematological examination.

Serum samples were collected by venipuncture of patients bitten by the snake *B. jararaca* (all the patients used in this study brought the snake for identification) and submitted to serum therapy in the Vital Brazil hospital at the Butantan Institute, São Paulo, Brazil. All the patients received a large amount of the specific antiserum (200 or 600 ml). Blood was collected immediately after the admittance of the patient to the hospital and at different times afterward. Blood coagulation was allowed to occur at room temperature and serum was obtained by centrifugation in a refrigerated centrifuge. The serum samples were either used immediately after collection or kept frozen at −20°C until used. *B. jararaca* reference venom prepared at the Butantan Institute was used. The ELISA technique for anti-venom detection (THEAKSTON *et al.*, 1977) was used. Antigens of *B. jararaca* venom that react with these antibodies were analysed by western blotting (TOWBIN *et al.*, 1979).

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Indeed, the increase in antivenom IgG antibodies was concomitant with a decline in the horse anti-venom antibodies (not shown). However, it is possible that the administration of the antivenom may have reduced or even abolished the antibody response due to sequestration of the immunogenic material in the circulation and/or to feedback inhibition induced by the horse anti-venom antibody or by the venom–antivenom complexes. This may explain the observation that 42% of the patients did not show a detectable antibody response. Our results also show that the specificity of the human anti-*B. jararaca* antibodies is very heterogeneous, each patient producing antibodies with different specificities. This heterogeneity may even be greater since conformational epitopes are not detected by the western blotting technique. It probably depends on the quantity and degree of absorption of each component of the inoculated venom as well as of the genetic background of the patients.

The persistent and high IgG antibody levels probably have a protective role since THEAKSTON *et al.* (1983) have observed that Nigerian victims of snake bite showed some resistance against subsequent snake bites. In addition, THEAKSTON *et al.* (1981) showed that sera from Ecuadorian indians bitten more than once by a poisonous snake were able to neutralize a lethal dose of the same venom. Our results suggest that this protective effect is due to antibodies of the IgG isotype.

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To determine the time of appearance and persistance of IgG antibodies anti-*B. jararaca* venom we analysed 36 serum samples collected at different times after the bite. Only 21 of these showed detectable IgG antibodies. The IgG antibody levels as detected by ELISA showed a very large individual dispersion as shown in Fig. 1. IgG antibodies appeared 18 days after the bite and kept increasing up to 80 days afterwards (Fig. 1). In patients bitten a second time, the IgG antibodies appeared sooner, about 3 days after the bite, attained higher levels than in the patients bitten only once and remained at a plateau up to the last day of serum collection usually between 60 and 80 days after the bite (Fig. 1). Furthermore, of 6 samples collected one year after the bite, one still had detectable IgG antibodies.

To determine the kinetics of IgM antibodies anti-*B. jararaca* venom we examined sera from 22 patients. Only 11 of these patients showed detectable but low IgM antibodies, in spite of the high levels of IgG antibodies present in the same patients. In general, IgM antibodies appeared sooner than IgG antibodies, about 3 days after the bite, and disappeared about 20 days after contrasting with the longer persistence of the IgG antibodies.

Figure 2 shows the results of the immunoblotting tests performed with sera from patients bitten by *B. jararaca*. The antibodies from each patient recognized different

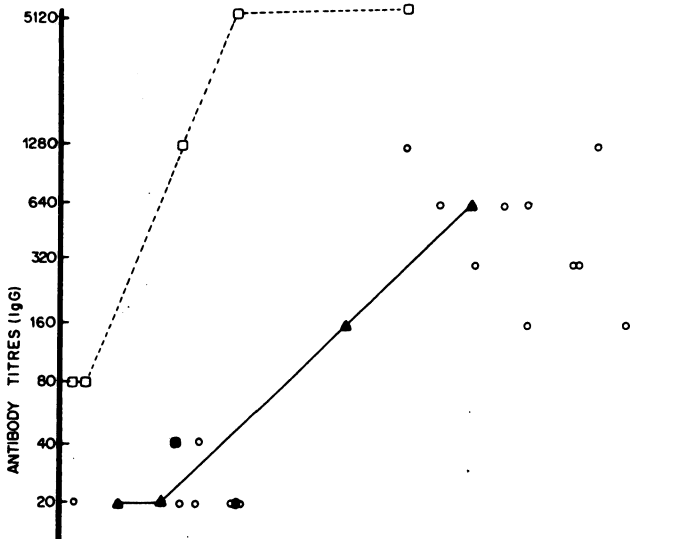

FIG. 1. DISTRIBUTION OF IgG ANTIBODY LEVELS IN PATIENTS BITTEN BY *B. jararaca* (○). Note the large individual dispersion. IgG antibody levels in a patient bitten once by *B. jararaca* (▲). Five other patients showed a similar primary antibody response. IgG antibody levels in a patient bitten a second time by *B. jararaca* (□). Note the earlier, faster and higher IgG antibody levels. Six other patients showed a similar secondary antibody response. Serum samples were assayed by ELISA at different times after the bite.

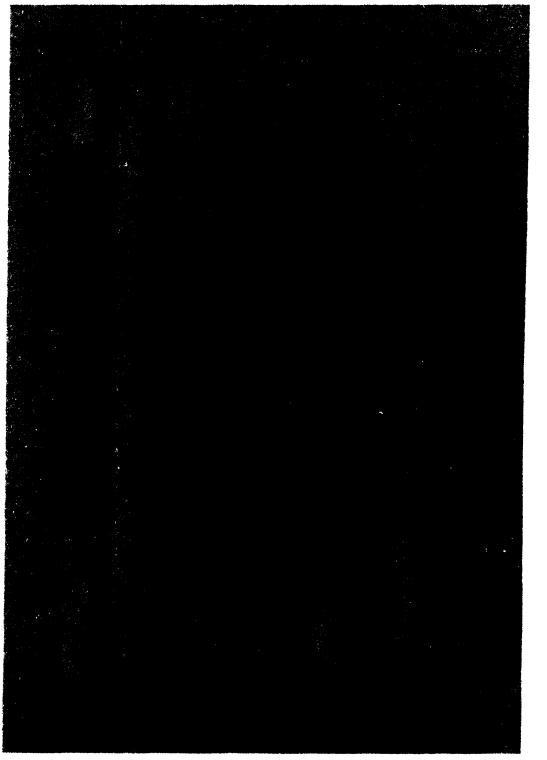

FIG. 2. *B. jararaca* VENOM WAS FRACTIONATED BY SDS-PAGE AND REVEALED BY WESTERN BLOTTIN(WITH ANTIBODIES FROM PATIENTS BITTEN BY *B. jararaca*.
(1), horse serum anti-*B. jararaca* venom; (2), normal horse serum; (3), patient serum with a ELISA titre of 640; (4), patient serum with an ELISA titre of 5120; (5), patient serum with a ELISA titre of 320; (6), patient serum with an ELISA titre of 1280; (7), patient serum with a ELISA titre of 160; and (8), normal human serum.

antigenic fractions in the *B. jararaca* venom. These differences do not seem to be (antibody content since a large excess of antibody was always used in the tests. Fu more, these differences were also not correlated with the antibody titer of sera instance, serum number 3 in Fig. 2 with an ELISA titer of 640, recognized 5 ant fractions, whereas serum number 6 in the same figure with an ELISA titer of 1280 sh only 3 antigenic fractions. The antigens in the bands of 50,000 to 60,000 mol. wt we most immunogenic. These results are very similar to those obtained in mice experime injected with *B. jararaca* venom in this laboratory (to be published).

It is interesting that in spite of serum therapy, the antibody response of the patie *B. jararaca* venom followed the classical humoral immune response pattern, show small and transitory IgM antibody response and a high and long lasting IgG ant response. Secondary response in patients bitten a second time consisted in an earlie higher IgG antibody response with no change in the IgM antibodies. The IgG ant response increased in time even though the patients received antivenom serum th