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A Study of Cellular and Humoral Immunity in Three Species of Armadillos. Part I.[1,2]

M. del C. Sasiain, E. D. Carosella, L. M. Balina,
D. M. Brezavscek and A. E. Bachmann[3]

Following the publications of Kirchheimer and Storrs ([6,7]) in 1971 and 1972, describing the dissemination of *M. leprae* in an armadillo (*Dasypus novemcinctus* Linn.), several papers reported the reproduction of leprosy in such armadillos after their inoculation with Hansen's bacilli obtained from nodules of lepromatous patients. Because of this and of the negative results following the inoculation of Hansen's bacilli in other experimental animals not previously immuno-depressed, it seemed of interest to carry out an immunologic exploration of other armadillos: *Chaetophroctus villosus* (Ch.v) *Dasypus hibridus septecinctus* (DHS), and *Zaedus Pichei* (ZP). These species are very primitive on the zoologic scale and have special hematologic characteristics such as hemoglobin which permits a high oxygen debit, as well as immunologic particularities such as the immune response to organ grafting involving the histocompatibility system ([1]). These findings indicate a difference in immunologic systems as compared with other experimental animals.

The purpose of the present investigation is the study of membrane receptors of immunocompetent cells as well as serum immunoglobulins, as a preliminary step for the future inoculation of these animals with Hansen's bacilli.

MATERIALS AND METHODS

Experimental animals. A total of 17 Ch.v, 6 DHS, and 1 ZP were used.

Separation of lymphocytes from peripheral blood. Samples of blood were obtained by cardiac puncture and diluted 1:2 in physiologic saline. They were then placed in a discontinuous gradient of Ficoll-Isopaque (1.074-1.060-1.050) in order to determine to which interphase the different cellular types corresponded ([3]). The appropriate density of the lymphoid population (1.074) was obtained following the technic of Trhosky *et al* ([12]).

Cellular immunity. Exploration of the surface markers of the peripheral blood lymphocytes was carried out in Ch.v, DHS and ZP. Receptors for sheep red blood cells (SRBC) or E rosettes: the lymphocytes with receptor for SRBC were identified following the technic of Jondal *et al* ([5]). Receptors for

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[3] M. del C. Sasiain, Ph.D., Master in Biochemistry; E. D. Carosella, M.D., Instituto de Investigaciones Hematológicas, Academia Nacional De Medicina de Buenos Aires, Las Heras 3092, Buenos Aires, Argentina; L. M. Balina, M.D., D.Sc., Professor of Dermatology, Facultad de Medicina Universidad del Salvador, Buenos Aires; D. M. Brezavscek, Technician; A. E. Bachmann, M.D., D.Sc., Chief, Immunohematology Laboratory, Instituto de Investigaciones Hematológicas, Academia Nacional De Medicina de Buenos Aires.

TABLE 1. *Cellular immunity.*

% Cells with receptors for:	*Chaetophroctus villosus*, nos. 1	2	3	4	5	mean	DHS	ZP
E	7	36.6	11	12.7	30	19.46	14	26.3
EAC	17	4.6	7	5	14.6	9.64	37.5	14.4
Ig-s	27	1.6	2.5	1.7	11	8.76	28	1.8
EA	0	0	0	0	0	0	0	0
"null cells"	49	57.2	79.5	80.6	44.4	62.64	20.5	57.5

the C3 complement fraction or EAC rosettes: the technic of Bianco *et al* (2) was followed. The lymphocytes were incubated with SRBC previously sensitized with rabbit hemolysin and fresh mouse serum complement.

Receptors for the Fc segment of immunoglobulins or EA rosettes: the technic of Froland *et al* (4) was followed incubating the lymphocytes with human RBC O Rh (+) (R1, R1) previously treated with anti-Rh serum (anti CDE).

The counting of E, EAC and Ea rosettes was carried out in a Neubauer chamber, considering positive those lymphocytes which had three or more RBC adherant to their membrane.

Receptors for surface immunoglobulins (Ig-s) were also investigated. Antiserum was obtained by immunizing three rabbits with a pool of serum from 17 Ch.v and three other rabbits with serum from three DHS, initially following the method of Proon (9), and later giving two serum injections together with complete Freund's adjuvant (one week of difference between the two injections).

The antisera obtained were labeled with fluorescein isothiocyanate, previously having precipitated the gamma globulin fraction with 50% ammonium sulfate. The purified lymphocytes were incubated with the different antisera following the technic of Rabellino *et al* (8, 10). The slides were observed under a Zeiss microscope by epi-illumination and phase-contrast, simultaneously.

Humoral immunity. Separation of the different gamma globulin fractions of the serum was done by passage through diethyl-amino-ethyl-cellulose (DEAE-cellulose) columns. For that purpose, 4 ml of a pool of Ch.v sera were dialyzed against phosphate buffer 0.005 M. It was loaded in the column and eluted with phosphate buffer. Protein concentration was read at 280 nm. The eluate was concentrated with PVP for micro-immunoelectrophoresis.

TABLE 2. *Humoral immunity.*

Phosphate buffer	No. of peaks	Fraction
0.005 M	1	$\gamma 2$
0.01 M	1	$\gamma 2$
	1	$\gamma 1$
0.3 M	2	$\alpha 2, \beta 2, \gamma A, \gamma 1$

TABLE 3. *Recycled $\alpha 2$, $\beta 2$, γA, $\gamma 1$ fractions.*

Phosphate buffer	No. of peaks	Fraction
0.015 M	1	$\gamma 1$
0.02 M	1	$\gamma 1$
0.03 M	1	$\gamma 1$
0.04 M	1	$\alpha 2, \beta 2$
0.05 M	1	γA

RESULTS

Cellular immunity. See Table 1.

Humoral immunity. The results by micro-immunoelectrophoresis of the different fractions isolated as compared with the guinea pig globulins are given in Table 2. The fractions $\alpha 2$, $\beta 2$, γA, and $\gamma 1$ were recycled with the following phosphate buffer concentrations: 0.015 M, 0.02 M, 0.03 M, 0.04 M, 0.05 M, and 0.1 M. The results were observed by micro-immunoelectrophoresis (Table 3, Figs. 1-3). The M fraction was obtained by passage through a Sephadex G-200 column eluting with buffer TRIS HCl 0.1 M/NaCl 1 M.

DISCUSSION

The results obtained in the studies on cellular immunity show that the lymphocytes of

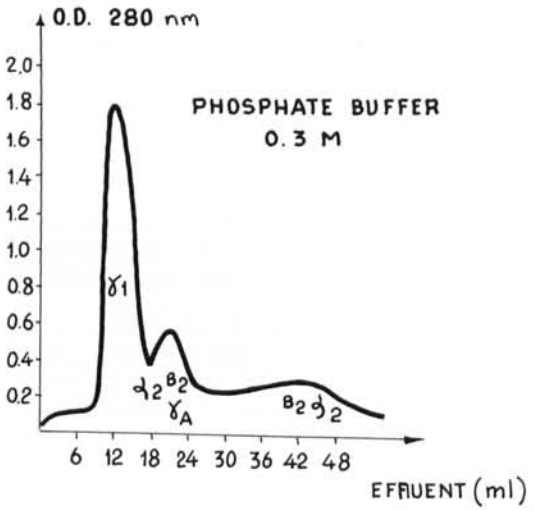

FIG. 1. DEAE-cellulose chromatography of *Chaetophroctus villosus* serum.

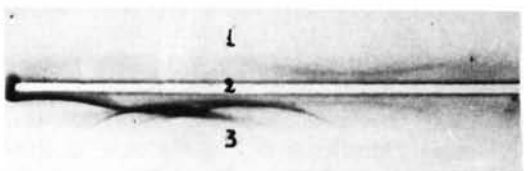

FIG. 2. Immunoelectrophoresis of Ch.v serum and 0.005 M phosphate buffer fraction. 1. 0.005 M phosphate buffer (γ2). 2. Ch.v. antiserum. 3. Ch.v. serum.

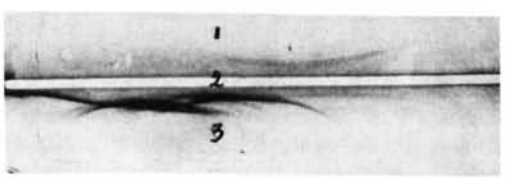

FIG. 3. Immunoelectrophoresis of Ch.v. serum and 0.3 M phosphate buffer fraction. 1. 0.3 M phosphate buffer (γ1). 2. Ch.v. antiserum. 3. Ch.v. serum.

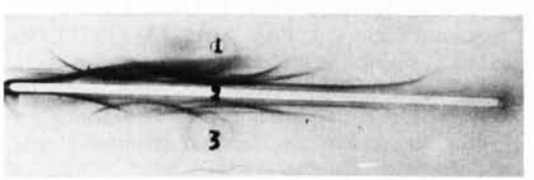

FIG. 4. Immunoelectrophoresis of DHS and Ch.v serum. 1. DHS serum (increased IgG). 2. Ch.v. antiserum. 3. Ch.v. serum (increased IgM).

the three species of armadillos studied have receptors for SRBC, EAC and Ig-s. It cannot be asserted whether the lymphocytes were T or B with the exception of those presenting Ig-s. Even though in man ([5]) the receptor for SRBC is characteristic for T lymphocytes, it cannot be so considered for T lymphocytes in species such as the mouse or the guinea pig. Cells bearing Ig-s may be considered as B lymphocytes, because in all species studied they are characterized by this presence of Ig-s ([11]). The EAC rosettes or receptors for C3 are characteristic of B lymphocytes. However, they are also present in tumor cells and macrophages ([11]).

In order to complete these determinations we will explore the central and peripheral lymphoid organs in the future.

With respect to humoral immunity, no qualitative alterations were observed in the different fractions obtained with the exception of a rise in IgM in Ch.v and IgG in DHS (Fig. 4).

From these studies it can be concluded that although the three species of armadillos present receptors for SRBC, C3 and Ig-s, they do not have Fc receptors. There is a high percentage of cells that do not have any type of receptors, "null cells," which may represent subpopulations of lymphocytes with unknown receptors. For the determination of such a possibility, immunologic exploration of the different lymphoid organs is necessary, extrapolating this study to the possible variations that may or may not be present in the inoculated animals or in those that may develop leprosy infection as well as any other alterations in humoral immunity.

SUMMARY

In the present study the membrane receptors of immunocompetent cells and immunoglobulins in three varieties of armadillos were explored for determining, in later studies, the possible differences in inoculated animals developing leprosy. The studies of cellular immunity were performed in five *Chaetophroctus villosus* (Ch.v), one *Dasypus hibridus septecinctus* (DHS) and one *Zaedus Pichei* (ZP), while the humoral immunity was studied with a serum pool of 17 Ch.v and 6 DHS. The results obtained demonstrate that the lymphocytes of the three species studied have receptors for SRBC, C3 and Ig-s, and no receptors for Fc segment of immunoglobulins. With reference to im-

munoglobulins no definite alteration of the humoral immunity was observed with the exception that DHS presents increased IgG levels and Ch.v increased IgM.

RESUMEN

Se estudiaron los receptores de membrana de las células inmunocompetentes, y las inmunoglobulinas, en tres variedades de armadillos con objeto de valorar, en estudios posteriores, las posibles alteraciones en los animales infectados con lepra. Los estudios de la inmunidad celular se hicieron en 5 *Chaetophroctus villosus* (Ch.v), un *Dasypus hibridus septecinctus* (DHS) y un *Zaedus Pichei* (ZP). La inmunidad humoral se estudió en una mezcla de sueros de 17 Ch.v y en 6 DHS. Los resultados obtenidos demostraron que los linfocitos de las tres especies estudiadas tuvieron receptores para SRBC, C3 e Ig-s, pero no tuvieron receptores para el fragmento Fc de las inmunoglobulinas. En relación a las inmunoglobulinas, no se encontraron alteraciones en la inmunidad humoral excepto que en DHS hubieron niveles elevados de IgG y en Ch.v se encontraron niveles elevados de IgM.

RÉSUMÉ

Au cours de cette étude, on a exploré les récepteurs sur les membranes de cellules immuno compétentes, de même que les immunoglobulines, dans trois variétés d'armadillos. Le but était de déterminer, en vue d'études ultérieures, les différences éventuelles que l'on pourrait observer chez des animaux développant la lèpre à la suite d'inoculation. Les études d'immunité cellulaire ont été menées chez cinq *Chaetophroctus villosus* (Ch.v), un *Dasypus hibridus septecinctus* (DHS), et un *Zaedus Pichei* (ZP). L'immunité humorale a été étudiée dans un pool de sérum provenant de 17 *Chaetophroctus villosus,* et de six *Dasypus hibridus septecinctus.* Les résultats obtenus montrent que les lymphocytes qui ont été étudiés chez ces trois espèces ont des récepteurs pour SRBC, C3 et Ig-s, mais qui n'ont pas de récepteurs pour le segment Fc des immunoglobulines. En ce qui concerne les immunoglobulines, aucune altération nette de l'immunité humorale n'a été observée, à l'exception de *Dasypus hibridus septecinctus* présente une augmentation des taux de IgG, et que chez *Chaetophroctus villosus* montre une augmentation des IgM.

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