

## ANTIGENIC VARIANTS OF THE SOLDADO VIRUS (*NAIROVIRUS*, *BUNYAVIRIDAE*) ISOLATED IN DIFFERENT PARTS OF THE WORLD

C. CHASTEL, F. LE GOFF, G. LE LAY

Virus Laboratory, Faculty of Medicine, 22 avenue Camille Desmoulins — BP 815,  
29279 Brest Cedex, France

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*Summary.* — Antigenic analysis of 9 strains of the Soldado virus, originating from seven different countries of Central and Northern America, Africa, Northern Europe and of the Pacific area was carried out by means of complement fixation (CF) and immunodiffusion (ID) tests. Three strains isolated from *Ornithodoros (A.) capensis*, including the reference Trinidad strain, were found to be related within the same relatively heterogeneous sub-group C, being characteristic for the New World. Five other strains isolated from *Ornithodoros (A.) maritimus* were classified to form another, much more homogeneous subgroup M, characteristic for the Old World (related to the Ireland strain). The South Africa strain, isolated from *Ornithodoros (A.)*, probably *capensis*, could not be classified in either of the above two subgroups. Thus, the Soldado virus in fact represents a complex of more or less closely related strains, but not a single virus.

*Key words:* Soldado virus; geographical distribution; antigenic variants

### Introduction

The Soldado virus (SOL) is a *Nairovirus* of the Hughes serological group, transmitted by ticks associated with seabirds. Its geographical distribution is very vast, affecting five of the six large zoogeographical regions of the world (Le Lay-Roguès, 1980; Chastel and Le Lay-Roguès, 1981).

It was isolated for the first time in 1963 (Jonkers *et al.*, 1973), from a mixed batch of *Ornithodoros (A.) capensis* Neumann and of *O. (A.) denmarki* Kohls, Sonenshine and Clifford, infesting a noddy (*Anous stolidus*) at Soldado Rock in the Caribbean Sea. It has subsequently been isolated on numerous occasions from *Ornithodoros* ticks belonging to the *capensis* complex, which parasitize seabirds and other birds (Le Lay-Roguès, 1980). In Africa, it is

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present in the Seychelles Islands (Converse *et al.*, 1975), in Ethiopia (Hoogstraal *et al.*, 1976b), in South Africa (Le Lay-Roguès, 1980), in Senegal (Main *et al.*, 1980) and in Morocco (Chastel *et al.*, 1981a). It has also been isolated in Texas, North America (King *et al.*, 1977), and in Wales (Converse *et al.*, 1976), Ireland (Keirans *et al.*, 1976), and France (Chastel *et al.*, 1979, 1980, 1981b). In the Pacific area the virus was detected in Hawaii (Yunker 1975). The SOL virus is potentially pathogenic to seabirds (Feare, 1976; King *et al.*, 1977; Le Lay-Rogues, 1980) and to man (Chastel *et al.*, 1981a).

The very wide geographical distribution, which is noteworthy amongst the arboviruses, but also the tri-segmented RNA genome (Clerx *et al.*, 1981) makes the SOL virus suitable for genetic recombinations; it was possible, therefore, to predict the existence of antigenic variants according to their geographical origin. Such variants are already known for a number of arboviruses: yellow fever, West Nile, Japanese encephalitis, Chikungunya, Sindbis, complexes of the viruses of American equine encephalitis, Californian encephalitis, Ťahyňa, etc... We attempted to demonstrate the existence of such antigenic variants amongst SOL virus strains of various geographical origin by means of CF and ID tests.

The results showed that such variants do exist indeed, implying that the SOL virus is not a single strain, but rather a complex of strains which are antigenically more or less closely related.

### *Materials and Methods*

*Nature and origin of the SOL virus strains.* The following informations are collated in Table I for the 9 SOL virus strains: nature of the strain, number of IC passages in mice, year of isolation, tick vector, region of geographical origin and reference. The standard Trinidad strain (TR 52214), was kindly provided by Dr. J. Casals, Y.A.R.U., New Haven, Connecticut, U.S.A. The Ireland, South Africa, Hawaii and Texas strains were kindly supplied by Dr. C. E. Yunker, R. M. L., Hamilton, Montana, U. S. A. All strains were adapted or readapted by one or two intracerebral (i.c.) passages to 24 hr-old newborn mice. The strains coming from Brittany (Chastel *et al.*, 1979, 1980, 1981b; Le Lay-Roguès, 1980) or Morocco (Chastel *et al.*, 1981a) were directly isolated in our laboratory by i.c. inoculation of newborn mice. None of the strains was cloned.

*Preparation of antigens and antibodies.* The antigens were prepared from the brains of infected young mice by the conventional sucrose-acetone method of Clarke and Casals (1958). Two of the immune mice ascites fluids, i.e. the anti-Ireland and anti-Texas, were prepared at the R.M.L. by C.E. Yunker. Immune sera to the other strains were prepared in adult mice by means of three intraperitoneal (i.p.) injections of living virus suspension in Freund's adjuvant at 10-day intervals. The serum was collected seven days after the last injection. The antigens and antibodies were kept in lyophilized form until used.

*Serological techniques.* The Kolmer's type of CF test with prolonged fixing in the cold (4 °C) was performed on microplates. Two units of each antigen strain, standardized beforehand, were introduced into successive ten-fold dilutions of homologous and heterologous antibodies to the 9 strains. The results were expressed as the reciprocal of the strongest dilution of antibodies still giving a positive CF reaction with two antigen units.

As to the ID test, the microtechnique of Chan (1965) was employed developed for the rapid typing of dengue-fever viruses. The antigens and antibodies were introduced undiluted into the wells. The assessment was made 5 days after diffusion, both by transillumination and by staining the precipitates with amido black. The results were noted according to the number of clearly identifiable precipitation lines.

Either the CF or ID tests were repeated two or three times.

Table 1. SOL virus strains compared in this study

Strain	Number of i.c. passages in mice	Year of isolation	Vector tick	Region of origin	Reference
Trinidad, Soldado reference strain	17	1963	<i>O. capensis</i> +	Soldado Rock, Caribbean Sea	Jonkers <i>et al.</i> , 1973
South Africa	3	1973	<i>O. capensis</i>	Lambert Bay, South Africa	Mc Intosh, pers. com., 1979
Ireland	10	1973	<i>O. maritimus</i>	Great Saltey Island, Ireland	Keirans <i>et al.</i> , 1976
Hawaii	9	1975	<i>O. capensis</i>	Manana Island, Hawaii, USA	Yunker, 1975
Texas	3	1975	<i>O. capensis</i>	Arcansas National Park, Texas, USA	King <i>et al.</i> , 1977
T13 Cape Frehel	5	1977	<i>O. maritimus</i>	Cape Frehel, Brittany, France	Chastel <i>et al.</i> , 1979
T101 Cape Frehel	5	1978	<i>O. maritimus</i>	Cape Frehel, Brittany, France	Chastel <i>et al.</i> , 1980
T234 Essaouira	3	1979	<i>O. maritimus</i>	Essaouira Island, Morocco	Chastel <i>et al.</i> , 1981a
T247	3	1979	<i>O. maritimus</i>	Cape Sizun, Brittany, France	Chastel <i>et al.</i> , 1981b

Table 2. Cross relationships of some Soldado complex viruses in the CF test

Antigen	Hyperimmune serum or ascitic fluid titre*								
	Trinidad	South Africa	Ireland	Hawaii	Texas	T13	T101	T234	T247
Trinidad, Soldado reference strain	<b>64</b>	8	16	128	1024	< 8	8	< 8	8
South Africa	8	<b>1024</b>	16	16	1024	16	64	< 8	32
Ireland	8	64	<b>1024</b>	64	1024	256	1024	512	1024
Hawaii	16	8	16	<b>1024</b>	1024	8	16	8	16
Texas	8	< 8	16	64	<b>1024</b>	< 8	32	< 8	8
T13 Cape Frehel 1977	4	16	1024	16	16	<b>256</b>	1024	512	1024
T101 Cape Frehel 1978	< 8	< 8	8	< 8	16	8	<b>128</b>	8	32
T234 Essaouira 1979	8	16	1024	16	128	16	1024	<b>64</b>	128
T247 Cape Sizun 1979	< 8	8	64	< 8	8	16	1024	16	<b>64</b>

\* Highest dilution of immune fluids fixing complement with two units of antigen.

### Results

#### Cross reactions of the SOL complex strains in CF test

The results of CF test were sometimes difficult to interpret because certain heterologous reactions were higher as the homologous ones; this is the case with the strains isolated at Essaouira (T234) and at Cape Sizun (T247). This type of unusual reaction seems fairly frequent with the viruses of the Hughes group (C. E. Yunker, pers. comm., 1981).

Table 3. Cross relationships of some Soldado complex viruses in the CF test

Antigen	Value of r* coefficient								
	Trinidad	South Africa	Ireland	Hawaii	Texas	T13	T101	T234	T247
Trinidad	1	0.03	0.04	0.17	0.35	< 0.01	< 0.01	< 0.01	< 0.01
South Africa		1	0.03	0.01	< 0.01	0.04	< 0.01	< 0.01	0.06
Ireland			1	0.03	0.12	1	0.25	1	1
Hawaii				1	0.25	0.02	< 0.01	0.04	< 0.01
Texas					1	< 0.01	0.06	< 0.01	0.03
T13 Cape Frehel 1977						1	0.5	0.70	1
T101 Cape Frehel 1978							1	1	1
T234 Essaouira 1979								1	0.70
T247 Cape Sizun 1979									1

\*  $r = \sqrt{r_1 \times r_2}$  ( $r_1$  = heterologous to homologous titre ratio of virus 1 with virus 2;  $r_2$  = heterologous to homologous titer ratio of virus 2 with virus 1)

Table 4. Cross relationships of some Soldado complex viruses in the ID test

Antigen	Hyperimmune serum or ascitic fluid								
	Trinidad	South Africa	Hawaii	Texas	Ireland	T13	T101	T234	T247
Sub-group C ( <i>capensis</i> )									
Trinidad	1*	1	1	1	—	—	1	1	1
South Africa	1	1	—	—	—	1	—	—	1
Hawaii	1	2	1	2	1	1	2	1	1
Texas	—	1	1	1	—	—	—	1	—
					15/16 = 0.93**		12/20 = 0.6		
Sub-group M ( <i>maritimus</i> )									
Ireland	—	2	1	2	2	1	2	1	1
T13 Cape Frehel 1977	1	—	1	—	1	1	1	1	1
T101 Cape Frehel 1978	1	—	—	—	—	1	1	—	1
T234 Essaouira 1979	1	1	—	—	—	—	1	1	—
T247 Fape Sizun 1979	—	1	—	—	—	1	1	1	1
					11/20 = 0.55		21/25 = 0.84		

\* Number of precipitin lines: all tests were performed twice. \*\* Ratio of the number of precipitin lines observed to the number of tests performed.

As follows from Table 2, the reference Trinidad strain is distantly related to all the other strains. On the other hand, the Ireland strain isolated from *O. (A.) maritimus* gives more distinct cross reactions with all the other strains, except Trinidad, in particular with the strains isolated from *O. (A.) maritimus* at Cape Frehel (T13, T101), at Essaouira (T234) and at Cape Sizun (T247). The South Africa strain isolated from *Ornithodoros*, probably *capensis*, is also albeit distantly related to the Ireland strain. Finally, the Texas and Hawaii strains are on the whole closer to the Trinidad strain.

Based on these results, we determined the 'r' coefficient in order to express the antigenic relationships between two viruses 1 and 2, according to the formula:

$$r = \sqrt{r_1 \times r_2}$$

in which  $r_1$  expresses the ratio of the heterologous to the homologous serum titre to the virus 1 and  $r_2$  expresses this ratio for virus 2. This ratio has already been used to compare different arboviruses (Yunker *et al.*, 1979) in particular to express the phenotypic relationships between different viruses of the Hughes group. If  $r$  was found  $> 1$  (because of a stronger heterologous than homologous reaction), it has been noted equal to 1 (Table 3).

Table 3 shows more clearly the antigenic relationships between various SOL strains:

1. The Texas strain and, to a lesser extent, the Hawaii strain, both isolated from *O. (A.) capensis*, are related, albeit distantly, to the reference strain

- Trinidad, itself isolated from a mixture of *O. (A.) capensis* + *O. (A.) denmarki*.
2. The Ireland strain, isolated from *O. (A.) maritimus*, appears to be the prototype of various strains isolated from the same ticks in France (T13, T101, T247) and in Morocco (T234).
  3. The South Africa strain, isolated from *O. (A.)*, probably *capensis*, cannot be clearly classified relative to the other strains.

#### *Cross relationships of the SOL strains detected by ID test*

The results seemed first aberrant to us or at least they were difficult to interpret. In fact, some heterologous reactions were entirely negative. Other heterologous reactions revealed two precipitation lines in contrast to only one in homologous reaction line. Thus, the ID test seemed to be of little value in assessing the antigenic relationships which can exist between various SOL virus strains.

Later on, we classified the ID lines a priori according to the results of the CF reactions into two subgroups: subgroup C comprising the strains isolated from *O. (A.) capensis* and subgroup M including the strains isolated from *O. (A.) maritimus*. It was then possible to demonstrate general but not individual differences between the two subgroups (Table 4). We calculated the ratio of the total number of precipitation lines observed to the total number of ID tested carried out in both C and M subgroups with respect to homologous and heterologous antibody reactions. It is clearly seen that this ratio is higher in the case of the homologous reactions (0.93 and 0.84) than in the case of the heterologous reactions (0.6 and 0.55). This is in favour to the existence of two antigenic subgroups of SOL strains, associated with their geography and, in particular, with the tick vector type (*O. (A.) capensis* or *O. (A.) maritimus*, as had already been suspected by Yunker *et al.* (1979).

#### *Discussion*

It is not surprising that we demonstrated the existence of antigenic variants amongst several viral strains previously classified as SOL virus or SOL-like virus. Such variants are known for a number of arboviruses and their existence for the SOL virus is supported by following findings:

— Antigenic differences which we observed between the reference strain Trinidad and two of our strains, T101 Cape Frehel 1978 and T234 Essaouira, Morocco, were confirmed at the Y.A.R.U. by Dr. J. Casals in CF and neutralization tests.

The Trinidad strain on the one hand and the Ireland-Texas strains on the other hand, can easily be separated by means of the immunofluorescence reaction by hyperimmune sera (C. E. Yunker, pers. comm., 1981).

Of greater interest is the fact that we were able to establish a clear separation between the strains originating from the New World, isolated from *O. (A.) capensis* and *O. denmarki*: Trinidad, Texas and Hawaii (subgroup C), and the strains originating from the Old World, transmitted by *O. (A.) maritimus*: Ireland, Cape Frehel 1977 and 1978, Cape Sizun 1979 and Essaoui-

ra 1979 (subgroup M). This separation according to the vector and the geographical distribution has a biological significance: it probably represents a process of selection by the vector.

In an evolutionary perspective, it could even be accepted that the seabirds which are the hosts of these ticks might have disseminated, over very large distances, the SOL virus strains which had been subject to segregation for hundreds of millions of years ago at the time of divergence of the various tick species today constituting the *Ornithodoros* (*Alectorobius*) *capensis* complex; in particular *O. (A.) capensis* and *O. (A.) maritimus* had diverged (Hoogstraal *et al.*, 1976a).

However, we were unable to assign the South Africa strain to one or another subgroup with the methods used, and not all strains isolated in the world have been examined. Valuable information could certainly be obtained by comparing the South Africa strain with strains which have been isolated in Ethiopia (Hoogstraal *et al.*, 1976b), in the Seychelles Islands (Converse *et al.*, 1975) and in Senegal (Main *et al.*, 1980), all of which originate from *O. (A.) capensis*. In addition, antigenic comparison of the strain of SOL virus isolated from *Amblyoma loculosum* in the Seychelles (Hoogstraal and Feare, 1982) would be of great interest. Furthermore the results we obtained with relatively less sensitive serological techniques should be refined by more sensitive methods, such as typing of the strains by means of monoclonal antibodies or analysis of the structural components of virions as it had just been carried out with the viruses of the Californian encephalitis complex (Klimas *et al.*, 1981; Said *et al.*, 1979).

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