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AMXFD ltr, 9 Feb 1972

TRANSLATION NO. 1128

DATE: July 1968

DEPARTMENT OF THE ARMY
Fort Detrick
Frederick, Maryland

INFLUENZA AND STAPHYLOCOCCUS INFECTION

Revue Belge de Pathologie et de Medecine Experimentale. Journal Tome XXVI, No. 6, Decembre 1858. Virus Grippal et Infection staphylococcique PAR Andre Dachy (Institut Pasteur de Bruxelles. Translation by Bernice MacDonald.

The favorable influence of infuenza virus on diverse bacterial infections responsible for pulmonary complications of human influenza has been demonstrated by P. Bordet and L. Quersin in the particular case of the Pfeiffer bacillus, the hemolytic strptococcus and of pneumococcus.

It has seemed interesting to study in this respect the staphylococcus pyogenes whose role in the complications of influenza, is becoming particularly frequent. It is proved rapidly that the guinea pig by reason of its weak receptivity to the staphylococcus infection do not lend themselves to this study. Sensitively very receptive, the rabbit was chosen as the experimental animal and we have recognized that the simultaneous inoculation of influenza virus and staphylococcus pyogenes very rapidly increases the receptivity of the rabbit to this bacteria.

The first strain of staphylococcus used has been isolated recently beginning with a case of purulent pleurisy and which manifested very strong resistance to the bacteriostatic action of penicillin. Also it appeared indicative of research for the use of other strains, if the important growth of the receptivity observed was not in proportion to the classic origin of the staphylococcus strain or to its resistance to penicillin. The experimental results have not opened any clear conclusion to

this subject.

I. General Experimental Conditions

The increase of receptivity in the animal to a bacterial infection is estimated according to the lowering of the minimum fatal dose of this bacteria when it is inoculated in an previously "influenzad".

Preparation of the suspension of the influenza virus: An allantoid suspension of influenza virus A-PR8 is concentrated by adsorption on human red corpuscles of RH+ following elution at 37°. This eluate of virus is titered by the method of hemagglutination defined by Hirst (2) and regularly yielding a titer between 8,000 and 16,000 hemagglutinat units per ml. The virulent liquid thus obtained contains traces of hemoglobin resulting from the slight hemolysis which was produced in the course of handling.

Preparation of the suspension of staphylococcus: In all the cases, the strains of experimental staphylococcus have not undergone anything except laboratory tests. The cultures in continuous beds on slopping gelose and aged 24 hours are collected in a physiological solution with 5% ordinary bouillon added. The required dilutions are realized extemporaneously.

The staphylococcus strains used possessed common characteristics required for the qualification of pyogenes, namely, the production of pigment, presence of a coagulant, fermentation of mannetal and early liquefication of the gelatine. The bacteriostatic antibiogrammes have been established according

to the disk method (Difco brand).

II EXPERIMENTS ON GUINEA PIGS

The guinea pig is a species of animal in practice resistant to the influenza virus. Its receptivity to staphylococcus pyogenes is moderate. The subcataneous injection involves the apparition of an abcess which evolves naturally toward the recovery (3). The death of the animal 24 to 48 hours can follow the intraperitoneal inoculation of high doses of staphylococcus.

The guinea pigs used, a total number of 47, weigh less than 335 gr. The inoculation done intraperitoneally allows a volume of 25 ml. of virulent eluat and 2 ml. of staphylococcus suspension.

The criterion adopted is the death of the animal after 4 hours.

RESULTS

1. Virus A-PR_8 + staphylococcus penicillino-resistant of pulmonary origin.

Dose of staphyl. in fraction of culture		1	1/3	1/5	1/10	1/20	1/100	1/1000
Test	Inoculated	5	6	2	4	2	-	-
	Deaths	5	4	0	0	0	-	-
A-PR8	Inoculated	-	2	2	4	3	3	1
	Deaths	-	2	2	1	0	0	0

The minimum fatal dose of virus A-PR8 for the guinea pig weighing 300 gr. is on the order of 160,000 hemagglutinat units.

weak staphylococcus engender a ftal pyemia in 4 to 10 days later with multiple revived abcesses.

Very rarely do they come from other visceral localizations: in the lungs, the the bones, the bone junctions. The hemocultures 24 hours after injection are irregularly positive.

Seventy one rabbits were used weighing less than 2,900 gr., the limits are between 1,970 and 3,250.

The criteria of virulence adopted is the death of the animal in 4 days.

The dose of influenza virus A-PR_8 is on the order of 20,000 hemagglutinat units and is inoculated under a volume of 2.5 ml. Injected alone, this dose of virus does not cause preceptible accidents. The suspension of staphylococcus pyogenes is contained in a volume of 2 ml.

In the guinea pig R. Bordet and L. Quersin (1) have shown that the size of the infection of virus and of bacteria is indifferent to the extent to which the two injections are not divided except by several hours. Also we have performed our injections almost simultaneoulsy.

The results were presented as follows:

A. Reduction of the minimum fatal dose of staphylococcus pyogenes injected intravenously under the action of influenza virus injected intratracheally in the particular cases following:

1) strain staphylococcus of pulmonary origin penicillinioresøstant.

2) strain staphylococcus of pulmonary origin penicillinosensetivø.

The minimum fatal dose of staphylococcus pyogene is for the same animal on the order of 1/3 of continuous culture.

These results indicate a slight increase of the receptivity to the bacteria, but the increase seems too weak to lend itself well for experimental study.

2. Virus A-PR$_8$ and staphylococcus pyogene, penicillino-sensetive of cutaneous origin.

The variation of receptivity stated in these tests, between "influenza" guinea pigs and test guinea pigs, do not appear to be superior to those observed in the preceding experiments.

CONCLUSION:

One can conclude that in the case of staphylococcus, the guinea pig does not lend itself as evidence of an increase of receptivity under the action of the influenza virus.

III. EXPERIMENTS WITH THE RABBIT

The influenza virus inoculated intratracheally proliferate in the rabbit if the animal is placed in a state of low resistance (4). Injected intravenously the weak quantities of influenza virus only cause a febrile response attaining its maximum in a few hours (5,6) showing the toxic effects of the virus. The rabbit, like the guinea pig, doesn't seem normally sensetive except to the toxic effects of the influenza virus.

On the other hand, the strong doses of staphylococcus given intravenously cause a rapid emaciation and the death of the rabbit of septecemia. after 2 to 3 days. The doses of very

3) Strain staphylococcus of cutaneous origin penicillino-resistant.

4) Strain staphylococcus of cutaneous origin penicillino-sensitive.

B. Comparison of the decrease of the minimum fatal dose of the staphylococcus pyogenes penicillino -resistant of pulmonary origin (intravenous or intratracheal).

1) Strain staphylococcus of pulmunary prigin penicillino- resistant.

Dose of staphyl. in fraction of culture		1/10	1/50	1/100	1/500	1/1000	1/10000
Test	Inoculated	1	1	1	1	2	-
	Deaths	1	0	0	0	0	-
+PR8	Inoculated	-	-	3	5	7	1
	Deaths	-	-	3	4	4	0

2) Strain staphylococcus of pulmonary origin penicillino-sensetive.

Dose of staphyl. in fraction of culture		1/10	1/50	1/100	1/500	1/1000
Test	Inoculated	1	2	1	-	-
	Deaths	1	1	0	-	-
+PR8	Inoculated	1	-	2	1	2
	Deaths	1	-	2	1	1

3) Strain staphylococcus of cuataneous origin penicillino-resistant.

Dose of staphyl. in fraction of culture		1/10	1/50	1/100	1/600	1/1000
Test	Inoculated	1	1	1	1	1
	Deaths	1	0	0	0	0
+PR8	Inoculated	-	1	1	2	2
	Deaths	-	1	1	1	0

4) Strain staphylococcus of cutaneous origin penicillino-sensetive.

Dose of staphyl. in fraction of culture		1/10	1/50	1/100	1/500	1/1000
Test	Inoculated	1	2	1	1	-
	Deaths	1	0	0	0	-
+PR8	Inoculated	1	1	3	2	3
	Deaths	1	1	1	1	0

These results regularly show an increase of receptivity to staphylococcus under action of the virus, but they do not indicate on the contrary any certain influence on this phenomenom, of the clinical origin of the bacterial strain nor of the sensetivity to penicillin.

The following table, which gives the sum of results obtained allows the greater estimate of the degree of this increase.

Dose of staphyl. in fraction of culture		1/10	1/50	1/100	1/500	1/1000	1/10000
Test	Inoculated	4	6	4	3	3	-
	Deaths	4	1	0	0	0	-

+PR8	Inoculated	2	2	9	10	14	1
	Deaths	2	2	7	7	5	0

The minimum fatal dose of staphylococcus pyogenes seems to be 20 times weaker when it is inoculated simultaneously with 20,000 units of influenza virus A-PR8.

C. Comparison of the decrease of the minimum dose of staphylococcus pyogene penicillino-resistant of pulmonary origin (method I.V.) as a function of the method of inoculation of virus (intravenous or intratracheal).

Dose of staphl. in fraction of culture		1/100	1/500	1/1000	1/10000
PR8 I. T.	Inoculated	3	5	7	1
	Deaths	3	4	4	0
PR8 I. V.	Inoculated	1	2	2	1
	Deaths	1	2	1	0

The means of inoculation of the virus (intravenous or intratracheal) therefore seem without influence on the degree of reduction stated.

D. Remarks

1. At the time of death a hemoculture by puncturing the cardiac is dosne systematically. In 50% of the cases death occurred 48 hours after inoculation, these hemocultures proved positive in the staphylococcus which had been injected simultaneously with the influenza virus or whether it wasn't.

2. The presence of influenza virus in the respiratory tree does not favor the pulmonary localization of the staphylococcus inoculated intravenously. There have been only several cases of pulmonary abcesses or pleurisy whose frequency does not exceed that pointed out in the literature (4) during the isolated inoculation of staphylococcus pyogenes given intravenously.

CONCLUSION

The receptivity of the rabbit to intravenous injection of staphylococcus pyogenes is received when one inoculates simultaneously 20.000 hemagglutinat units of influenza virus administered intratracheally.

This increase of sensitivity is independant of the means of inoculation of the virus, of the sensitivity of strains of staphylococcus to penicillin and of the clinical origin of the stain.

The viruses do not multiplu in the rabbit and the effects are immediate, the increase of receptivity can be attributed to a toxic effect of the virus.

The presence of virus in the respiratory tract of the rabbit does not entail the very frequent apparition of staphylococcus abcesses at this level.

SUMMARY

Influenza virus, when injected simultaneously, slightly increase the receptivity of guinea pigs to Micrococcus ~~pyogenes.~~

Intratracheal or intravenous inoculation of influenzal virus increases at least 20 times the receptivity to the infection of rabbits inoculated intravenously with suspensions of staphylocci.

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