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LEIDEN - HOLLAND

No. 9

STUDIES ON THE ANTIGENIC COMPOSITION OF INFLUENZA VIRUS B STRAINS

with the aid of
the haemagglutination inhibition technique

by

L. M. BRANS

Introduced by

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CONTENTS

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INTRODUCTION

FRANCIS (1940) and MAGILL (1940) were the first to discover influenza virus B strains in the year 1940, and subsequently all workers who have isolated B strains have found their antigenic composition to deviate more or less from the classical strain LEE (1940, U.S.A.). References on this subject are given by TAMM et al. (1950). DUDGEON et al. (1946) state that some pairs of patients' sera, obtained during the influenza B epidemic in the winter of 1945—'46, both on the Continent and in England, showed no rise in antibody titre in the haemagglutination inhibition test against the LEE strain, although a rise was observed against a strain which was isolated during the epidemic (strain CRAWLEY). Other pairs of sera gave a more marked rise against the epidemic strain than they did against LEE. They found three B strains, isolated in 1945—'46, all very similar to one another and also very closely related to the B strains: ELIZ (1945, Austr.) and MIL (1945, Austr.) isolated in Australia. A further strain, isolated in London in 1943 (PADDINGTON (1943, Eng.)) behaved differently. Antisera obtained from ferrets were used. TAMM et al. (1950), employing rabbit sera, compared 9 B strains, isolated in different years, with the LEE strain; these strains proving to deviate greatly from the latter. They also noted slight mutual dissimilarity in antigenic pattern, and this led them to speak of a continuous spectrum of antigenic differences in the influenza B group.

Isolation of influenza B strains in Holland. During the A-prime epidemic in the winter of 1949 we isolated one B strain among 45 A-prime strains. In the spring of 1950 7 B strains were isolated.

Our experiences with the first 6 serum pairs in patients, titrated against the LEE strain, (haemagglutination inhibition test), were identical with those of DUDGEON et al. in 1946. Three out of the 6 serum pairs showed a very slight or no increase at all in titre against this strain, and a distinct increase against a B strain isolated during the epidemic (table 1). As the non-specific

inhibition had been eliminated by treating the sera with a crude filtrate of *Vibrio Cholerae*, these results leave us in no doubt regarding the difference in antigenic composition between the strains IERS (1950, Ned.) and LEE. Since this difference was so great as to interfere with the use of the LEE strain as a reference strain for the serological diagnosis of influenza B, we next compared a number of B strains with regard to their antigenic composition, employing the haemagglutination inhibition technique.

INVESTIGATIONS

Materials and methods. Viruses. Cross tests were first performed with a total of 26 B strains, but later 3 more strains were examined, bringing the final number to 29. The passage formulae are given in the tables. Nearly all strains were inoculated in the amniotic cavity of 13 day old chick embryos, the allantoic route being used for the strains LEE (1940, U.S.A.) and ROHA (1950, Ned.). After two days' incubation the amniotic (or allantoic) fluid was pooled, centrifuged and diluted with physiological saline in the ratio 1: 4. Merthiolate was added to give a final dilution of 0.01 %. The pooled fluid was stored at 2° C. Not all crossings could be performed with the fluid from one pooling. We aimed constantly at keeping the dissimilarities in passage formulae of antigens and corresponding antisera as small as possible.

Ferret antisera. Anaesthetized ferrets were inoculated intranasally with 1 ml. egg-fluid possessing a minimal C.C.A. titre of 240 (0.5 % final dilution of chicken red cells). The serum was collected after 12 days. All pre- and post-infection sera were checked for the presence of antibodies against strains from the A, A-prime and B groups. The immune sera were stored at 2° without adding preservative. A portion of each serum was freeze-dried.

Elimination of the non-specific inhibition in ferret sera with enzyme of Vibrio Cholerae. (VAN DER VEEN and MULDER (1950)). Enzyme of *Vibrio Cholerae* was prepared according to BURNET and STONE (1947). A good production of enzyme was obtained with the strain 4 Z (kindly sent to us by Prof. F. M. BURNET). As a standard for a good enzyme production we have found that two parts of crude filtrate added to one part of normal ferret serum, after 16 hours' interaction at 37°, should eliminate every non-specific inhibition from this serum, when tested against the strains A (1941, Ned.); A-prime-BARRATT (1947, Eng.); B-Co (1950, Ned.), and B-TODD (1950, Eng.). We have found, that when a filtrate in this dilution eliminates the non-specific inhibition in ferret serum against the strain A-prime BARRATT, it is always sufficiently potent to eliminate the non-specific inhibition against B strains. In our cross tests one part of antiserum was treated with 5 parts of crude filtrate for 16 hours at 37°. After this period the mixture was heated for one hour at 56° to remove the remaining of R.D.E. (BURNET and STONE (1947)). In each experiment a normal, filtrate treated serum, known to contain a large quantity of non-specific inhibitor, was titrated against all the strains used in this experiment. The titre of this normal treated ferret serum was always less than 12 against all B strains tested in the investigations. Any possibility of residual non-specific inhibition, therefore, is excluded in all the experiments.

Haemagglutination inhibition test. This test was performed by means of a micro-method (tiles of porcelain with concavities). The technique used and the estimation of the end-point were identical to those described by VAN DER VEEN and MULDER (1950). All the experiments were performed with great care by ourselves. The standard deviation of the titration method may be estimated at $\pm$ 15 % (VAN DER VEEN and MULDER (1950)).

The problem of crossing egg-lines and egg-mouse-egg-lines of influenza virus with the haemagglutination inhibition test. This problem is important in the B group since most strains are pure egg-lines. Ferret-mouse-egg-lines are the strains LEE (1940, U.S.A.), MONTGOMERY (1940, U.S.A.) and TM (1940, U.S.A.). The strain PADDINGTON (1943, Eng.) is a ferret-egg-line. Crossings of B strains, isolated after 1940 with the classical LEE strain are crossings between egg-line and ferret-mouse-egg-line virus.

Results of crossings between egg-lines and egg-mouse-egg-lines of strains of influenza virus of the A and A-prime groups have been published by us elsewhere (MULDER and BRANS (1952)). From this investigation it became clear that we have to make allowance for the fact that antisera against egg-mouse-egg-lines often show low, and even extremely low, titres against homologous and/or heterologous egg-lines from the same subgroup. The same phenomenon may occur in tests with anti-LEE serum against egg-lines of other B strains. This also holds good for the antiserum of the strains MONTGOMERY (1940, U.S.A.), TM (1940, U.S.A.), and PADDINGTON (1943, Eng.).

Cross-tests. Antisera of 10 strains from the period 1940—1948 were crossed, each antiserum being titrated against all the 10 strains in one experiment (table 2). In a second series the antisera of 15 strains, from the period 1949—1950, were crossed in the same way (table 3). From the results of these tests we selected 6 strains (isolated in different years), for a complete cross test with all the other strains. We selected those strains which gave the impression of being serologically separate, and those which showed good homologous and heterologous antititres: LEE (1940, U.S.A.); BON (1943, Austr.); PADDINGTON (1943, Eng.); WARNER (1948, Austr.); BUD 1 (1949, Hung.), and TODD (1950, Eng.). We added the strain CRAWLEY (1946, Eng.), (DUDGEON et al. (1946)), which was not available at the time of the preliminary crossings of tables 2 and 3. This strain happened to show high homologous and heterologous antititres, and fitted with the selected group very well.

Fresh ferret antisera were employed for the crossings with the 7 above mentioned strains. Some antigens had to be made over again. Tables 4 and 5 show the results of the crossings of the 7 strains above mentioned with all the other strains. Tables 6 and 7 show the results of the mutual crossings between 7 and 5 selected strains, carried out in a single experiment.

From tables 4, 5, 6 and 7 the following pecularities of the B strains examined come to light.

Discrepancies in the tests. As the crossings of the selected strains were repeated several times on different days, partly with the original, and partly with fresh antisera, one could expect certain discrepancies, to occur.

A serious discrepancy was found in the crossings of the antiserum of BON (1943, Austr.) with the strain PADDINGTON (1943, Eng.). Serum Bon E_{42} gave high titres with the strain PADDINGTON, while serum E_{47} gave a low titre. Table 8 shows a repetition of the crossings of the two strains and here again the antiserum BON E_{47} yielded a low titre with PADDINGTON: the use of a different anti-BON-serum must account for this discrepancy. The dissimilarities of the heterologous titres of the antisera CRAWLEY E_{15} and E_{14} against the strains WARNER and TODD were probably also due to the use of two different sera. Another discrepancy concerned the homologous titres of the strain GOODLOE, which was determined twice with the same antiserum, yielding widely differing results. The heterologous titres of the antiserum against the strains LEE, BON, PADDINGTON and WARNER on the other hand, agreed very well in the two tests. In this case a titration error might have been present. The strain BERKELEY 1 (1949, U.S.A.) had a low homologous titre in the tests as shown in table 4. In the tests of table 3 the homologous titre was found to be higher, although the same antiserum and the same virus-pool were used. It is just possible that there was a technical error in these titrations too. The same may be said about the tests with the strain BERKELEY 2 (1949, U.S.A.).

Strains with low homologous titres. Some strains are poor antigens, their homologous titres being low. This is particularly true of the strains isolated in 1949 and 1950. Of some strains the homologous titres are very low, and certain heterologous titres higher (BERKELEY 2 (1949, U.S.A.); SLU (1950, Ned.); HES (1950, Ned.)). They may be considered to represent Q-phase strains (VAN DER VEEN and MULDER (1950)). Tests with the

strain BUD 1 (1949, Hung.), however, teach us to use care in considering a strain to be a Q-phase strain, for a second antiserum may show a much higher homologous antititre.

Serological patterns in the B group. Strain Lee (1940, U.S.A.). From the tables 1, 2, 4, 5, 6 and 7 it is quite obvious that the strain LEE stands alone, a fact, which is convincingly proved by the low titres of this strains against heterologous antisera. In order to make sure that this is not due to this strain being a ferret-mouse-egg-line, the strain was crossed with the egg-line and the egg-mouse-egg-line of the strain BON (1943, Austr.)[1]). Tables 9 and 10 show that the mouse-adapted lines of these two strains also show distinct antigenic differences. The only strain of near relation to LEE is the strain MONTGOMERY (1940, U.S.A.), isolated by EATON and BECK (EATON and BECK (1941)) (tables 2, 11 and 14). It is curious that the strain also shows a high titre with the serum CRAWLEY (1946, Eng.) and reacts fairly strongly to the serum $F_xE_xE_3$ PADDINGTON (1943, Eng.). The strain, therefore, shows a greater polyvalence against heterologous antisera than LEE.

The strain Paddington (1943, Eng.). This strain, isolated by Dr F. HIMMELWEIT in London (HIMMELWEIT (1943)), seems to have a separate place in the series. The high heterologous titre of this strain with one of the antisera BON (1943, Austr.) is striking.

The group Bon (1943, Austr.). It is an important feature that the remaining strains, isolated after 1943, may all be reasonably considered to be strongly related to the strain BON (1943, Austr.). The strain CRAWLEY (1946, Eng.) behaves almost identical to BON, except in the behaviour of its antiserum against the strain MONTGOMERY.

The strain TM (1940, U.S.A.). This strain, isolated by Dr T. P. MAGILL (MAGILL (1940)), and only examined at a later date, has been proven to be an independent (tables 12, 13 and 14).

Identification of B strains isolated in 1951 in Holland. These strains were isolated in the spring of 1951 by Prof. Dr J.D. VERLINDE in Amersfoort (Holland), when the investigation mentioned above had already been completed. Both strains may be incorporated in the subgroup BON-CRAWLEY (tables 15 and 16).

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[1]) DR E. HERTZBERGER (Weesp) kindly sent us the mouse adapted line of the strain Bon.

DISCUSSION

One surprising result of the present investigation is the fact that, among 29 B strains, we have found only one related to the classical strain LEE (MONTGOMERY (1940, U.S.A.)). It is moreover noteworthy that a third strain isolated in 1940 in America (TM) stands alone as does a strain isolated in England in 1943 (PADDINGTON). The remaining number of the strains investigated (25), may, with a reasonable degree of certainty, be classed together in a single group, the first representative of which would seem to be the strain BON, isolated by BEVERIDGE, BURNET and WILLIAMS in 1943 in Melbourne (BEVERIDGE et al. (1944)). This strain is a good antigen, and it gives high titres with heterologous antisera from the same subgroup. The strain is therefore very suitable as a reference strain for this group. The chance that (for instance) the strain TM may diverge from LEE because both underwent many animal passages, is, in our opinion, very slight. In the case of A and A-prime strains, mouse passage tends to make those strains serologically more homogeneous (MULDER and BRANS (1952)); moreover the ferret-mouse-egg-line of the strain MONTGOMERY (1940, U.S.A.) is very closely related to LEE. Although the total of the serological B variants found does not differ from that of the A strains (among which we distinguish nowadays 4 subgroups, viz. A-swine, A-WS, A-PR_8, and A-prime), yet the B group has the pecularity that more than one subgroup was isolated in a single year (1940 and 1943), which does not seem to have been proven for the human A-group so far (ISAACS and ANDREWES (1951)) and that no other representatives of the strains TM and PADDINGTON were found. Since 1943, however, the antigenic pattern of the B strains seems to have undergone little or no change.

With regard to the problem of vaccination against influenza B, we would be inclined at present to replace the strain LEE by some more recently isolated strain; or perhaps the strain BON,

or Crawley. It should be remembered that good results were reported by Francis et al. (1946) on vaccination with the strain Lee in an influenza B epidemic (1945). This could be explained by the assumption that vaccination with the strain Lee stimulated residual antibodies to the Bon group which produced a titre high enough to prevent this type of strain from causing infection.

SUMMARY

Twenty nine B strains isolated in the period 1940—1951 were examined with regard to their antigenic composition with the aid of the haemagglutination inhibition technique, ferret antisera being employed. Four more or less separate patterns of subgroups were found, viz. LEE (1940, U.S.A.), TM (1940, U.S.A.), PADDINGTON (1943, Eng.) and BON (1943, Austr.). Only one strain was closely related to LEE (MONTGOMERY (1940, U.S.A.)). No other representatives were found of the strains TM and PADDINGTON.

Twenty five strains could reasonably be considered as belonging to a single subgroup of strains, of which the strain BON (1943, Austr.) seems to have been the first representative discovered. Q-phase strains were infrequent among the 29 strains that were examined.

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GENERAL INDEX TO THE TABLES

The nomenclature for each strain is that used by the Centre from which the strain was obtained. The year of isolation and the country of origin are added. Austr. = Australia; Czech. = Czechosl. = Czechoslovakia; Eng. = England; Hung. = Hungary; Ned. = The Netherlands; U.S.A. = United States of America.

In the passage formulae the letter F, M or E indicate the number of ferret-, mouse- or egg-passages. So, $F_8M_{137}F_{160}$ implies that the strain had 8 ferret-, 137 mouse- and 160 egg-passages. F_x, M_x or E_x implies that the number of previous ferret-, mouse- or egg-passages is unknown.

The inhibition titres were calculated theoretically, and are expressed as reciprocals of the final serum dilution which produced a partial agglutination (50 %) when 3 A.U. of virus were used.

TABLE 1

Haemagglutination inhibition tests with 6 pairs of human sera (1950) against strains of influenza virus (one experiment)

PAIRS OF SERA (Human)	STRAINS			
	A (mouse adapted) PR_8 (1934, U.S.A.)	A-prime (egg) Heer (1949, Ned.)	B (mouse adapted) Lee (1940, U.S.A.)	B (egg) Iers (1950, Ned.)
Iers	<12/<12	96/96	<12/18	<12/108
Wou	168/168	72/72	<12/20	<12/256
Slu	<12/<12	<12/<12	<12/<12	<12/1024
Liesh	80/80	160/144	<12/1792	<12/1792
Pugf	320/320	<12/<12	<12/746	21/1194
Hesk	72/72	21/21	<12/1024	<12/384