Prevention of Herpes Genitalis by the ‘Bulgarian’ Vaccine F.HSV-2v (PRK): Preliminary Clinical Evidence

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Aim. To ex am ine the an ti genic prop er ties of the for mal in-in ac tivated her pes sim plex vi rus type 2 (HSV-2) vi rus-par ticle vac cine F.HSV-2v (PRK), which has been used ther ape u tically in Bul gar ia for 30 years, and to make pre lim i nary as sess ment of its po ten tial protec tive ef ficacy by a fol low-up of vac ci nated pa tients with her pes geni talis.

Methods. Prop erties of the vac cine were ex am ined by stan dard im mu no log i cal lab or a tory tests. Fifty-five pa tients at risk of her pes geni talis re ceived 2-4 vac ci na tions and were mon i to red dur ing a 6-year fol low-up.

Results. The vac cine was an ti genic in lab or a tory tests and ab sorbed neu tral iz ing an ti body from hy per im mune rab bit se rum against her pes sim plex vi rus type 1 (HSV-1). In vac ci nated pa tients, there was an over all con trac tion rate of her pes geni talis of 5.4%. There was no ev i dence of sig ni ficant lo cal or gen er al ized ad verse ef fects from vac ci na tion.

Conclusion. Bul gar ian vac cine F.HSV-2v (PRK) may have protec tive ef ficacy, which, in as so ci a tion with its ap par ent safety from our find ings and from its clin i cal use for over 30 years in Bul gar ia, sug gests that it should be scruti nized by a for mal clin i cal trial.

Key words: ad ju vants, im mu no log i cal; Bul gar ia; her pes geni talis; her pes labialis; HIV; sex be hav i or; sex u ally trans mit ted dis eases; vac cine

Her pes vi rus in fec tions con tinue to be a ma jor prob le m, with rates of con trac tion of in fec tion ris ing alarm ingly in the past 20 years. A re cent re view by Kinghorn (1) in di cates that, while rates of other sex u ally trans mit ted dis eases such as gon or rhea and sy philis have de clined, the inci dence of her pes geni talis in the United King dom has in creased three fold. Cur rent pat terns of sexual be hav i or sug gest that this trend will con tinue in the fore see able fu ture. Her pes geni talis can cause con sid er able dis tress and de bil i ta tion and has been linked to sus cep ti bil ity to other in fec tions – in par tic u lar the hu man im mu no de fi ciency vi rus (HIV) – with in volve ment in the pro gres sion of dis ease through, ac ti va tion of HIV tran script ion (4).

Med i ca tions such as Acyclovir or Valacyclovir have been only par tially suc cess ful in the treat ment of her pes sim plex in fec tion. Fur ther more, there is a pos si bil ity of drug re sis tan t strains and as so ci a ted prob lems, i.e., sup pres sion of the host im mune sys tem dur ing long term ther a pie (5). Addi tionally, drug treat ments seem un able to re du ce con trac tion of in fec tion in part ners of symp to mat ic car ri ers who are not re ceiv ing drug treat ment. There are no re ports of pro tec tive ef ficacy in hu mans of any vac cines cur rently un der de vel op ment.

While there is a prog ress made in the search for an ef fec tive and safe pre ven ta tive vac cine, suc cess ful tri als of pro tec tive ef ficacy of any vac cine cur rently un der de vel op ment have not yet been re ported. The pre ven ta tive ef ficacy of ‘Skin ner’ in ac ti vated intra cel lular vac cine, which has been in named pa tient us age in the United King dom for 2 de cades (6) and has un der gone pla ce bo-con trolled trial of ther a pie ef ficacy in the United States (7), has not yet been scruti nized in pla ce bo-con trolled tri als due to the lack of fund ing. Sub unit glyco pro tein prep a ra tions and re com bi nant glyco pro teins have been un success ful in the treat ment of her pes sim plex in fec tion. Fur ther more, there is a pos si bil ity of drug-res is tan t strains and as so ci a ted prob lems, i.e., sup pression of the host im mune sys tem dur ing long term ther a pie (5). Addi tionally, drug treat ments seem un able to re du ce con trac tion of in fec tion in part ners of symp to mat ic car ri ers who are not re ceiv ing drug treat ment. There are no re ports of pro tec tive ef ficacy in hu mans of any vac cines cur rently un der de vel op ment.

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cover pathogenicity through com bination with wild type strains—a matter of se rious im portance for a vi rus with po ten tial to cause keratitis or en cephalitis. It is im pera tive, there fore, not to for go the eva luation of all re as on able vac cine can did a tes.

We have eval uated a vac cine, de sign a ted as F.HSV-2, (PRK), in ac cor dance with the no men clature we have used for our vac cine de ives ve loped in the UK dur ing the pre vi ous two de cades (15-18). The vac cine is for ma lin-inac tivated (F) and pre pared from 5 strains (,) of HSV-2 iso lated from pri mary rab bit kid ney cells (PRK). Al though this vac cine has been in clin i cal prac tice for the the re a per tic ular man age ment of her pes oph the ral mic us in Bul gar ia for 30 years (19-24), its pre va lentative ef fi cacy has never been in vest i gated. We re port on the pre limi nary evi dence of pre va lentative ef fi cacy in pa tients of pa tients with her pes ge neti cals.

Materia ls and Meth ods

Vac cine

The Bul gar ian vac cine is a for ma lin-inac tivated vi rus par ti cle pre par a tion ob tained from cellu lar ex tracts of her pes sim plex vi rus (HSV-2) in fected new born rab bit kid ney cells (NCIPD Dried Her pes vac cine type II, Na tional Cen ter of In fec tious and Para sitic Dis eases, Sofia, Bul garia). The vac cine vi rus strains in clude 5 field iso lates – which were orig i nally se lected from 400 newly iso lated strains with dif fer ent pro perties for their im munogenic abil ity and ab scence of on copo ten tial or of eff ects on chro mosomes (19-21,23,25). Af ter iso la tion, these strains were lyoph ilized at ter 2-4 pas sages. Vac cine vi ruses used in this study were pre par ed from these pri mary pas sages, and the prepa ra tion has been de scribed else where (21,25). Briefly, the 5 vi rus strains were used to in fect roller cul tures of cells ob tained by try psiniza tion of kid ney cells from 10 to 14-day-old rab bits bred from Bra ty ryx lar ve and for immunogenicity and tox ic ity in mice and ham mar, UK). Se rum was re moved, trays were washed five times in PBS (Ox oid) con tain ing 1% Mar vel milk pow der (Cadbury Sch weppes Ltd., Bir mingham, UK). Se rum di lu ted in PBS/Tween/Mar vel ve loped for 1 h at 37°C and then re moved. The plates were washed as de scribed and in cu bated with peroxi dase-conju gated she ep anti she ep iso gens (45 U/l d i luted 1:1,000 or 1:2,000 in PBS/Tween/Mar vel ve loped with PBS at 37°C for 1 h. Then, the plates were washed once in PBS and se rum di lu tions were ad min is ter ed for 1 h at room tem per a ture. The re veal ment was re moved, trays were washed five times with PBS, and 121-I-labeled pro tein A ad died at 30,000-30,000,000 con cts per min (cpm) per well. Af ter cuta na liza tion at 37°C for 1 h, the plate was washed and dried, cut in to di m di a tural wafers, and counted in a Lum i na cam mera.

Ab sorption of Neu tral i zation

The abil ity of vac cine to ab sorb neu tral iz ing an ti body from hy per im mun e vac cine was ad min is ter ed by sub cuta ne ous in oc u la tion into the pos ter ior as se teral (30,31) in all but 5 cases, where ad ju vant was not used but the vac cine was main tained in BHK-21 cells. Each vac cine dose (Batch 460288) con tained at least 10 viral part icles.

Laboratory Anal y sis of the Vac cine

An ti genic ity of the vac cine was ad min is tered in our lab or a tory in the UK by poly acrylam ide gel electrophore sis, im munodif fus ion, ELISA, radioimmunoas say, and ab sorp tion of neu traliz ing an ti bod es. There fore, the pa tients ex pe ri enced nor mal con di tions of ex po sure. There were not asked to adopt con tra ceptive or other bar rier meth ods of des ease pre ven tion that usu ally ad vanced in clin i cal prac tice. There fore, the pa tients ex pe ri enced nor mal con di tions of ex po sure to her pes ge neti cals, but whose sex ual his tory of des ease was re vealed and 1-4 vac cine pas sages and the vac cine was pre par ed against the ‘Skin ner’ vac cine NFU.Ac.HSV-1[S Bul gar ian HSV-1 vac cine F.HSV-1 com pared against the ‘Skin ner’ vac cine NFU.Ac.HSV-1[S Bul gar ian HSV-2 vac cine F.HSV-2

Poly acrylam ide Gel Elec tro phoresis (PAGE)

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was the dose used in pre vi ous stud ies with *herpes ophthal micus* in Bul gar ia (23,24).

Pa tients were asked to make im me di ate con tact in the event of any sus pected out break of *herpes ge nitalis* and were fol lowed up by in ter view and phys i cal ex a mina tion in ter vals of 3 months over the first year after vac ci na tion. There af ter, pa tients were fol lowed up by tele phone in ter view.

**Results**

**Prop erties of Vac cine**

The pro tein com po si tion of the vac cine was ex amined by polyacylamide gel elec tro pho re sis (PAGE); there were polypeptides in mo le cular weight re gions of ap prox i ma tely 60-70 kD, 100-150 kD, and 200-250 kD (data not shown). There were no immu noprecip i tins on test ing with anti-HSV type 1 or type 2 hy perim mune rab bit bi ser, as ex pected in a non-disrupted vi rus par ticle vac cine.

On test ing by ELISA against hy perim mune anti-HSV-1 serum, anti gen dilu tion end points of log\(_{10}\) 2.3 and 1.8 were ob tained for the type 1 and type 2 vac cines, re spectively. End points of log\(_{10}\) 3.4 and 2.8 were ob tained on test ing against anti-gD (Band II) serum. Thes e val ues were slight ly lower than those ob tained with the ‘Skin ner’ herpes sim plex vac cine (32) against anti-HSV1 anti ser um, al though val ues against anti-gD (Band II) were com par a ble and pre sum ably re flected the rel a tive in crease in the pro por tion of glyco protein D in a

### Table 1. Socio-demographic features of patients

<table>
<thead>
<tr>
<th>Feature</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of subjects</td>
<td>55</td>
</tr>
<tr>
<td>Number of men</td>
<td>26</td>
</tr>
<tr>
<td>Age (years)</td>
<td>16-59</td>
</tr>
<tr>
<td>MeanSD</td>
<td>31.8±1.3</td>
</tr>
<tr>
<td>Social class( ^b )</td>
<td></td>
</tr>
<tr>
<td>A/B</td>
<td>32</td>
</tr>
<tr>
<td>C</td>
<td>17</td>
</tr>
<tr>
<td>D/F</td>
<td>3</td>
</tr>
<tr>
<td>Marital status</td>
<td></td>
</tr>
<tr>
<td>Not married</td>
<td>37</td>
</tr>
<tr>
<td>Married</td>
<td>16</td>
</tr>
<tr>
<td>Unrecorded</td>
<td>2</td>
</tr>
<tr>
<td>Ethnic background</td>
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</tr>
<tr>
<td>White European</td>
<td>47</td>
</tr>
<tr>
<td>Asian</td>
<td>5</td>
</tr>
<tr>
<td>African Caribbean</td>
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</tr>
<tr>
<td>Nationality</td>
<td></td>
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<td>UK</td>
<td>49</td>
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<td>USA</td>
<td>4</td>
</tr>
<tr>
<td>Republic of Ireland</td>
<td>1</td>
</tr>
<tr>
<td>Australia</td>
<td>1</td>
</tr>
<tr>
<td>History herpes labialis</td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>19</td>
</tr>
<tr>
<td>Negative</td>
<td>34</td>
</tr>
<tr>
<td>Unknown</td>
<td>2</td>
</tr>
</tbody>
</table>

\(^a\) Numbers do not tally to 55 if the required in formation was not known or not recorded for any of the patients.

\(^b\) Ac cording to the Reg is ter Gen er al’s So cial Scale, 1990, for the UK ("ABC1" scale): A – pro fes sional work ers (law yers, doc tors etc.), sci en tists, man ag ers of large scale or gan i za tions; B – shop keep ers, farm ers, teach ers, white-collar work ers; C – 1) skilled man u al (i.e., hand) work ers – high grade, e.g., mas ter build ers, car pen ters, shop as sis tants, nurses, 2) skilled man ual – low grade, e.g., elec tric ci anis, plumb ers; D – semi-skilled man ual, e.g., bus driv ers, lorry driv ers, fit ters, E – un skilled man ual, e.g., gen er al lab our ers, bar men, port ers.

**Clinical Study**

None of the 55 pa tients who de nied the study were fol lowed up by phys i cal ex a mina tion and phys i cal ex a mina tion at in ter vals of 3 months over the first year after vac ci na tion. There af ter, pa tients were fol lowed up by tele phone in ter view.

This study has ex am ined the ef fi cacy of a whole vi rus vac cine in pa tients at risk of con tract ing her pes vi rus in fec tion. The vac cine de monstrated an ti geni city in immu no log i cal as says and by ab sorp tion of HSV-1

**Table 2. Reactivity (Log\(_{10}\) endpoint reactivity) of Bulgarian vac cine and ‘Skin ner’ vac cine by ELISA**

<table>
<thead>
<tr>
<th>Serum</th>
<th>Bulgarian vac cine HSV Type 1</th>
<th>HSV Type 2</th>
<th>‘Skinner’ HSV vaccine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti HSV-1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1/50</td>
<td>2.1</td>
<td>1.9</td>
<td>3.8</td>
</tr>
<tr>
<td>1/300</td>
<td>2.4</td>
<td>1.7</td>
<td>3.8</td>
</tr>
<tr>
<td>Anti gD (Band II)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1/20</td>
<td>4.3</td>
<td>3.1</td>
<td>–</td>
</tr>
<tr>
<td>1/50</td>
<td>3.5</td>
<td>3.5</td>
<td>3.1</td>
</tr>
<tr>
<td>1/300</td>
<td>3.0</td>
<td>1.8</td>
<td>2.7</td>
</tr>
</tbody>
</table>

\(^a\) Re sults are ex pressed as end points of re ac tiv ity of a di lu tion se ries of the vac cine.
and HSV-2 neutralizing activity from hyperimmune rabbit sera.

The overall rate of disease contract rate in vaccinated patients was 7.2%, but only 4% in the vaccinated with adjuvant. This represents a marginally higher failure rate than the 2% contract rate in patients immunized with the Skin ner intracellular vaccine (6), where the majority of patients vaccinated with adjuvant may be sur pris ing in the light of studies by Dundarov et al (22-24), but hardly in di cates an essential require ment for adjuvant, as there were too few patients in this group for proper sta tis tical eval uation. How ever, pend ing fur ther evidence, there seems little reason to eschew Al hydrogel, which is safe, eco nom i cal, and has stood the test of time (30,31).

A retrospective analysis of vaccine a cquired herpes genitalis patients who had no clinical evidence of de novo infection in patients vaccinated with adjuvant. This is an open study and raises the critical question of the importance of virus. i.e., cellular DNA in vaccine preparations. Whereas it would seem gen er ally pru dent to min i mize virus DNA content in a po ten tial vaccine, and per haps more so if there is ev idence that this virus may have oncogenic po ten tial (35-37), there seems to be lit tle ev idence to sug gest that the in oc u lar inoculation of killed whole-virus vac cines has been accompanied by significant side effects (38). Indeed, there is no clear associ ation be tween herpes labialis and car i noma in that site, where the sub ject has pre sumably pre ferred to not have re cur rences and there would normally be less virus excretion in the asymptomatic a cquisition than during re curences. How ever, this impor tant issue could be resolved only by daily virus isolation, which, al though te dious and time-consum ing, is nev er less a feasi ble project.

There was no ev idence of side ef fects in any of the patients vaccinated with adjuvant. This is an open study and raises the critical question of the importance of virus. i.e., cellular DNA in vaccine preparations. Whereas it would seem generally pru dent to minimize virus DNA content in a potential vaccine, and perhaps more so if there is evidence that this virus may have oncogenic potential (35-37), there seems to be little evidence to suggest that the in oculo inoculation of killed whole-virus vaccines has been accompanied by significant side effects (38). Indeed, there is no clear association between herpes labialis and carcinoma in that site, where the subject has presumably preferred not to have recurrences and there would normally be less virus excretion in the asymptomatic acquisition than during recurrences. However, this important issue could be resolved only by daily virus isolation, which, although tedious and time-consuming, is nevertheless a feasible project.

Contraction rate in patients vaccinated with adjuvant may be surprising in the light of studies by Dundarov et al (22-24), but hardly indicates an essential requirement for adjuvant, as there were too few patients in this group for proper statistical evaluation. However, pending further evidence, there seems little reason to eschew Alhydrogel, which is safe, economical, and has stood the test of time (30,31).

A retrospective analysis of vaccine-acquired herpes genitalis patients who had no clinical evidence of de novo infection in patients vaccinated with adjuvant. This is an open study and raises the critical question of the importance of virus. i.e., cellular DNA in vaccine preparations. Whereas it would seem generally prudent to minimize virus DNA content in a potential vaccine, and perhaps more so if there is evidence that this virus may have oncogenic potential (35-37), there seems to be little evidence to suggest that the in oculo inoculation of killed whole-virus vaccines has been accompanied by significant side effects (38). Indeed, there is no clear association between herpes labialis and carcinoma in that site, where the subject has presumably preferred not to have recurrences and there would normally be less virus excretion in the asymptomatic acquisition than during recurrences. However, this important issue could be resolved only by daily virus isolation, which, although tedious and time-consuming, is nevertheless a feasible project.

There was no evidence of side effects in any of the patients vaccinated with adjuvant. This raises the controversial question of the importance of virus. i.e., cellular DNA in vaccine preparations. Whereas it would seem generally prudent to minimize virus DNA content in a potential vaccine, and perhaps more so if there is evidence that this virus may have oncogenic potential (35-37), there seems to be little evidence to suggest that the in oculo inoculation of killed whole-virus vaccines has been accompanied by significant side effects (38). Indeed, there is no clear association between herpes labialis and carcinoma in that site, where the subject has presumably preferred not to have recurrences and there would normally be less virus excretion in the asymptomatic acquisition than during recurrences. However, this important issue could be resolved only by daily virus isolation, which, although tedious and time-consuming, is nevertheless a feasible project.
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tients over 30 years in Bul garia, pro vides an ar gu-
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sub je cted to the scrui nity of for mal clin i-cal trial.

Acknowledgment
We thank Dr S. Dundarov, De part ment of Vi rology, Na tional
Center of In fec tious and Par a si tic Dis eases (NCIPD), So fia, Bul garia,
for sup ply ing us with the vac cine. The Vac cine Re search Trust, Bir-
ings ham, UK, it was cul tually sup ported the study.

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in fec tion as a risk fac tor for human im mun o de fi ciency vi rus in
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Haseltine WA, et al. Ac ti va tion of hu man im mu no de fi nition of
5 Mosca JD, Bed narik DP, Raj NB, Rosen CA, Sod ros ki JG,
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Received: April 5, 2000
Accepted: September 4, 2000

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