G SUMMARY

Molecular characterization of human infective and non-infective *Trypanosoma* (*Trypanozoon*) *brucei* isolates from south-east Uganda.

A wide range of animal reservoir hosts occurs for the two trypanosome subspecies infective to man, *Trypanosoma brucei rhodesiense* and *T. b. gambiense*. Morphologically, these two subspecies are not distinguishable from a third one, *T. b. brucei*, which does solely infect animals. Only a partial differentiation is possible by biological, biochemical or molecular biological methods. Therefore, quick and reliable test systems are required, which identify animals as carriers of human infectious trypanosomes.

In this study the PFGE karyotype patterns of 16 *T. brucei* reference stocks/clones (5 *T. b. brucei*, 9 *T. b. gambiense* und 2 *T. b. rhodesiense*), as well as 18 field isolates and 7 clones from animals and 4 field isolates from Rhodesian sleeping sickness patients were compared regarding their human serum sensitivity/resistance. The field isolates have been isolated between February 1990 and July 1992 in Bulutwe, Mukono district, south-eastern Uganda, where Rhodesian sleeping sickness is known to be endemic.

The *in vitro* HSRT as well as the *in vivo* BIIT (8 selected field isolates) were used to determine the resistance of *Trypanosoma brucei* bloodstream forms to human serum. The reference stocks showed human serum sensitivity (*T. b. brucei*) or resistance (*T. b. gambiense*) in the HSRT as expected. Of the *T. b. rhodesiense* reference stocks, one was strongly resistant but the other one sensitive. However, unequivocal HSRT results of *rhodesiense* stocks can be explained by loss of human serum resistance after subpassaging or cultivation in absence of human serum. Of the 18 field isolates from pigs and cattle in Bulutwe, 3 were resistant, 6 subresistant and 9 sensitive in the HSRT. The 7 clones showed human serum sensitivity like their origins. Of the 5 animal isolates selected for BII-testing, 3 were resistant and 2 subresistant. These results were only partially identical with earlier BIIT-investigations, supporting the fact that *T. b. rhodesiense* can loose human serum resistance. The 4 stocks isolated from Rhodesian sleeping sickness patients were human serum resistant, either in the HSRT or the BIIT (3 selected ones), as expected.

To figure all trypanosome chromosomes in a range from 95kbp to 3Mbp, a Biometra Rotaphor® type V PFGE unit (Biometra, Göttingen, D) was used applying four different PFGE running conditions (0-III). Good PFGE results were achieved by using $2x10^9$

trypanosomes/ml PSG buffer to prepare agarose blocks and lysing them *in situ* in the blocks with 1mg proteinase K/ml NDS buffer for 48h. Chromosome bands were visualized by staining with 1 µg/ml ethidium bromide (EtBr) for 30 min and destaining over night. Gels were photographed on a UV light box, the pictures edited in the documentational program *BioDoc*® (Biometra, Göttingen, D) and the banding patterns analysed by ScanPack 3.0® cluster analysis program (Biometra, Göttingen, D).

The stability and reproduceability of PFGE karyotype patterns were confirmed by analysing clones and their origins as well as bloodstream forms and procyclics or different VATs. A 100% similarity was observed. These findings implicate that trypanosomes do not change their karyotype during cyclical or variant antigen type development and that clones exhibit the same karyotype as their origin.

The reference stocks and clones exhibited extremely diverse banding patterns both in terms of size and numbers of chromosome-sized DNA molecules. In the region of mini- (MC) and mega-base (MBC) chromosomes all reference stocks displayed almost the same number of bands (4-7 MC, 4-6 MBC). Only the number of intermediate chromosomes (IC) varied, with *T. b. gambiense* showing less (0-4 IC) than non-*gambiense* stocks (3-6 IC). However, none of the *T. brucei* subspecies formed a separately clustered group within the dendrogram. The reference stocks were distributed among the different branches with low similarities between them, implicating that no subspecies differentiation is possible according to the PFGE results.

The banding patterns of the Bulutwe field isolates from pigs and cattle appeared to be more homogeneous, some of them even identical, as far as analysed. They showed a higher level of similarity than the human field isolates, whose banding patterns were as different as those of the references. However, similar or identical karyotypes of different stocks could only be related to an identical isolation date or animal. The grouping of the trypanosome stocks and clones analysed (I-V) according to their PFGE banding patterns does not allow a differentiation into human serum sensitive or resistant stocks, with the exception of group IV containing only human serum resistant *T. b. gambiense* stocks. Additionally, no single band correlated with the human serum sensitivity/resistance of a stock could be determined.

The PFGE method is easy to learn and carry out. It can specifically characterize trypanosome stocks and is able to detect even minimal changes in their genotype. Nevertheless, no differentiation into human serum sensitive or resistant trypanosome stocks, nor into the 3 subspecies was possible with respect to the karyotypes. The reason for this is the sensitivity of

PFGE lying at the level of chromosome size changes and therefore being relatively low. In trypanosomes, those size changes appear very often due to spontaneous gene-rearrangements.

To enlarge the sensitivity of the method, restriction enzyme digestion of chromosomes is suggested. Even more sensitive are the PCR methods, although they contain a high risk of contamination.