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General position statement of the ZKBS
on the classification of genetic engineering operations with
highly pathogenic avian influenza A viruses (HPAIV) which
possess the potential for efficient airborne transmission between mammals

Highly pathogenic influenza viruses (HPAIV) are influenza A viruses of the subtypes H5 and H7 which possess multiple basic amino acids at the cleavage site of the hemagglutinin (HA) and have an intravenous pathogenicity index of 1.2 or greater in 6-week old chickens [1]. HPAIV occur mainly in poultry stocks and cause major economic losses due to their high lethality. However, since 1997, over 600 laboratory-confirmed human infections with H5N1 with a lethality of 60 % have also occurred. Outbreaks of H7N7 in humans have so far been associated with a much lower lethality (ca. 0.2 %) [2]. Human infections with HPAIV usually occur as a result of transmission of the virus from infected poultry to humans. Sporadically, human cases have also been traced back to human-to-human transmission; however, sustained human chains of infection are not known to exist. In accordance with § 5 (1) of the GenTSV [Genetic Engineering Safety Regulations] HPAIV of the subtypes H5 and H7 are assigned to **risk group 3**.

In September 2011, at the 4th Meeting of the *European Scientific Working Group on Influenza*, experiments were presented in which a H5N1 virus was altered by genetic modification in combination with serial passaging in ferrets in such a way that the virus became transmissible from one animal to another via aerosols [3]. Ferrets are considered the best animal model for predicting the transmissibility of influenza viruses between humans. The media as well as the expert community reacted to this publication with a broad-based discussion about the benefits and risks of these experiments. Fears that the genetically modified viruses could be misused or escape inadvertently from the laboratory were expressed. On the other hand, it was pointed out that the experiments were essential both for the assessment of the pandemic potential of circulating HPAIV and for the development of vaccines. In January 2012, out of consideration for the public debate, a group of international influenza virologists agreed to a 60-day moratorium to suspend experiments on the transmission of HPAIV [4]. In May and June 2012, respectively, the experimental data from the studies conducted by the research groups led by Ron Fouchier at the Erasmus Medical Center in Rotterdam and Yoshihiro Kawaoka at the University of Wisconsin-Madison, USA on which the debate was based were published in full [5,6]. Just recently, the end of the moratorium and the resumption of studies were announced [7]. This raises the question of how genetic engineering operations with HPAIV are to be assessed in future. In the Netherlands, the Commission on Genetic Modification (COGEM) considers safety measures corresponding to biosafety level 3+ (e.g. use of Class III microbiological safety cabinets or Class III isolators in addition to level 3 safety measures) to be sufficient for this type of work. In Canada, biosafety level 4 was specified for operations with H5N1 viruses that possess the potential for efficient human-to-human transmission by the Public Health Agency of Canada.

In Germany, the Central Committee on Biological Safety (ZKBS) provides position statements to the Federal Government and to the competent authorities of the federal states (*Länder*) on questions relevant to safety in genetic engineering in accordance with § 5 of the GenTG [Genetic Engineering Act]. In particular, it advises on the safety classification of genetic engineering operations and the required safety measures for genetic engineering facilities. According to the precautionary principle formulated in § 7 (1a) GenTG, genetic engineering operations are to be assigned to the higher biosafety level, if there is any doubt about which level of safety is appropriate for the proposed genetic engineering operation. Moreover, the ZKBS classifies (wild-type) microorganisms as donor or recipient organisms for genetic engineering operations into risk groups according to § 5 (1) GenTSV. As required by § 5 (6) GenTSV, after consulting the ZKBS, the *Bundesministerium für Ernährung, Landwirtschaft und Verbraucherschutz* [German Federal Ministry for Food, Agriculture and Consumer Protection] regularly publishes a list of legal classifications of microorganisms. All of the studies published to date on HPAIV that are potentially transmissible by aerosols are genetic engineering operations [5,6]. Specific enquiries from authorities of the federal states have prompted the ZKBS to provide a general position statement on the classification of HPAIV which have the potential for efficient airborne transmission between mammals. Furthermore, the *Gesellschaft für Virologie* [Society for Virology] has expressly endorsed a risk assessment of the work on ferret-adapted HPAIV by the ZKBS [8].

Scientific background

The objective of the studies conducted by Ron Fouchier and Yoshihiro Kawaoka was to determine the evolutionary potential of HPAIV for efficient airborne transmission. They examined which and how many mutations are necessary to achieve airborne transmission in the ferret model. To do so, the two research groups followed different experimental approaches.

Using reverse genetics, Ron Fouchier's group first inserted two mutations into the HA of a H5N1 isolate (Q222L/G224S, numbering according to amino acid position in the mature HA), for which there is a known link with a switch in receptor specificity from the avian (α2,3-linked sialic acid) to the human type (α2,6-linked sialic acid) [5]. In addition, the E627K mutation, which is likewise already known, was inserted into the viral polymerase subunit PB2. This mutation is associated with higher replication efficiency in mammals. However, only after ten passages in ferrets under selective pressure for airborne transmission the modified H5N1 virus was capable of transmission from ferret to ferret via aerosols. Two further mutations were found in the HA of aerosol-transmissible viruses that had been generated in this way – H103Y in the trimerisation domain and T156A, which leads to the loss of a glycosylation site. Ferrets infected with the recombinant H5N1 virus via aerosols exhibited lethargy, loss of appetite and ruffled fur, but they did not die from the infection.

In Yoshihiro Kawaoka's experiments, first random mutations were inserted into the globular head of the HA of a H5N1 isolate and subsequently screened for adsorption to α2,6-linked sialic acid [6]. Two mutations were identified (N220K/Q222L) which cause a switch from avian to human type receptor specificity. Subsequently, using reverse genetics, a reassortant virus was generated with the recombinant H5N1 HA and the remaining seven genome segments of H1N1v (PB2 contains two mutations for adaptation to replication in mammals). By passaging in ferrets under selective pressure for airborne transmission the reassortant virus acquired two more HA mutations – N154D, which also causes the loss of the glycosylation site, and T314I, which affects the stability of the HA. This modified virus was efficiently transmitted between ferrets via aerosols. The infected ferrets exhibited weight loss of < 10 % and lesions in the lung, but they did not die from the infection.

Recommendation

According to § 5 (1) of the GenTSV in conjunction with the criteria listed in Annex I of the GenTSV, HPAIV of the subtypes H5 and H7, which possess an increased potential for efficient airborne transmission between mammals, as donor and recipient organisms for genetic engineering operations are allocated to **risk group 4**.
Genetic engineering operations in which HPAIV (**risk group 3**) are used as donor or recipient organisms to generate viruses capable of efficient airborne transmission between mammals as well as the handling of viruses generated in this way are to be assigned to biosafety level 4 according to § 7 paragraphs 1, 3 (no. 4) and 4 (no. 4) of the GenTSV in conjunction with § 7 paragraph 1(a) of the GenTG. Such genetic engineering operations can include, for instance, the targeted insertion of mutations into the genome of HPAIV, which lead to efficient airborne transmission between mammals, or the insertion of some of these mutations in combination with an adaptation of the recombinant viruses to appropriate animal models (e.g. ferrets or guinea pigs) by serial passaging with selective pressure on efficient airborne transmission.

Grounds

The objective of the above-described experiments by Ron Fouchier and Yoshihiro Kawaoka was to produce HPAIV that can be spread between ferrets via airborne transmission. So far, ferrets are considered the best animal model for predicting the transmissibility of influenza viruses between humans. Consequently, as a precaution it must be assumed that efficient airborne transmission of the genetically modified viruses can take place between humans. There is no pre-existing immunity to HPAIV of the subtypes H5 and H7 since they are not circulating in the population [5]. A recombinant HPAIV with the potential for efficient human-to-human transmission would therefore strike an immunologically naïve population. The average lethality for human H5N1 infections is approximately 60 %. Even though airborne transmissibility in the ferret model was associated with a reduced pathogenicity in the studies carried out to date [5], it is not possible to assess whether *(i)* this applies also to other HPAIV and *(ii)* these viruses would also be less pathogenic in humans. Hence, high pathogenicity of the genetically modified viruses for humans cannot be ruled out. Whether vaccination with a pre-pandemic H5N1 vaccine also confers immune protection against the recombinant viruses and whether the antiviral therapeutic agents against influenza viruses are also effective against the recombinant viruses is not known. It has in fact been shown that sera from humans or ferrets who had been immunized with a pre-pandemic H5N1 vaccine reacted with reassortants in which the HA, and in some cases also PB2, were derived from H5N1 and possessed the above-described mutations (furthermore, where applicable, insertion of a monobasic cleavage site in the HA) and the remaining genome segments originated from the laboratory strain A/Puerto Rico/8/34 [5,6]. In addition, in initial *in vitro* tests the recombinant HPAIV were sensitive to Oseltamivir [5,6]. However, a prophylaxis or therapy with medically proven efficacy is not available.

Note

For operations with wild-type HPAIV aiming at efficient airborne transmissibility between mammals but not involving genetic engineering steps the ZKBS also considers compliance to level 4 safety measures according to the BioStoffV [Biological Agents Ordinance] necessary, on the same scientific grounds as mentioned above.

References

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