Acta Microbiologica et Immunologica Hungarica, 48 (2), pp. 143–146 (2001)

ALTERNATE ADENOVIRUS TYPE-PAIRS FOR A POSSIBLE CIRCUMVENTION OF HOST IMMUNE RESPONSE TO RECOMBINANT ADENOVIRUS VECTORS

I. NÁSZ, ÉVA ÁDÁM, ANNA LENGYEL

Institute of Medical Microbiology, Faculty of General Medicine, Semmelweis University Budapest, Nagyvárad tér 4, H–1089 Budapest, Hungary

(Received: 21 March 2000; accepted: 5 April 2000)

With the help of monoclonal antibodies the existence of at least 18 different earlier not known intertype (IT) specific epitopes were demonstrated in different numbers and combinations on the hexons of different adenovirus serotypes. The IT specific epitopes play an important role in the experimental gene therapy and in the recombinant adenovirus vaccination because of the harmful immune response of the recipient organisms directed against the many different epitopes of the adenovirus vector. For the elimination of harmful effect the authors suggest the use of multiple vectors, each prepared from different adenovirus serotypes showing the loosest antigenic relationship to each other. The vectors would be used sequentially when second or multiple administration is needed. For this purpose the authors determined and described 31 such adenovirus type-pairs, which are probably the best alternates for sequential use in experimental gene therapy.

Keywords: adenovirus, antigenic relationship, recombinant adenovirus vector

Recombinant adenoviruses (rAdV) have been widely used as gene delivery vectors to target cells in experiments both with curative and preventive purposes. Adenovirus vectors have been used in the experimental gene therapy of genetic disorders, in immuno- and molecular therapy of a variety of cancers. Recombinant adenoviruses appear to be attractive candidates for vaccination against the infectious diseases, too. However, one of the major obstacles to their use is the strong cellular and humoral immune response of the host to the vector proteins. Neutralizing and non-neutralizing anti-vector antibodies may substantially inhibit the effect of recombinant adenoviruses especially when multiple administrations are required [1–4]. The explanation could be based on the complex antigenic structure of the hexon, the major coat protein of adenovirus, on which besides the genus- and type specific epitopes the

1217-8950/2001/$ 5.00

existence of numerous different intertype (IT) specific epitopes were demonstrated with monoclonal antibodies [5], and with prediction methods. The antigenicity and immunogenicity of synthesized peptides representing potential IT specific epitopes were also demonstrated [6]. It can be supposed that these IT specific epitopes play a significant role in the immune reactions both *in vitro* and *in vivo*. This prompted us to analyse the data concerning the epitope structure of the hexon and to determine the type-pairs showing the loosest antigenic relationships between each other.

Table I

31 alternate adenovirus type-pairs to develop recombinant vectors for sequential use in gene therapy

Adenovirus types	4,19 cluster I	8,9,9/13,10 cluster II	1,2,5,6 cluster III	7	12	35	13	26	41	18	27	SadV 16	BadV 2
12			6/11				5/10						
27				3/15	3/10	4/8							
35			5/13				5/10						
41		6/11		5/12	4/9	4/9							
BAdV2		6/11	5/11	3/15	3/10	4/8		4/10	4/7				
BadV3	1/16	1/15	0/15	1/13	1/8	1/8	0/10	1/12	0/9	1/10	0/8	1/11	1/6

The epitope content of each two hexon types are shown at meeting points: first number represents the number of identical and the second one the number of the different epitopes

Methods

For the investigation of epitope composition of different adenovirus hexon types 61 mouse ascitic fluids containing monoclonal antibodies (MAbs) have been used. MAbs have been developed in three panels directed against purified hexons of human adenovirus (HAdV) types 1, 35 and bovine adenovirus (BAdV) type 2, respectively. The distinction and marking of the different epitopes recognized by the MAbs have been carried out by the determination of the composite cross-reactivity pattern, the titer in ELISA and the correlation coefficient of all the 61 MAbs with 21 different hexon types representing all the six human subgenera, as well as different bovine and simian adenoviruses (SAdV) (see on Table I, upper horizontal row). The IT specific epitopes

defined by MAbs have been characterized by two groups of adenovirus serotypes: on which they are and on which they are not present [6]. The antigenic structure of the individual hexon types have been characterized by the determination of their IT specific epitope spectrum. The degree of antigenic relationship in the pairwise analysis has been expressed by the number of the identical and different epitopes present on any two given hexon types [5].

Results and discussion

The three panels of MAbs have recognized 22 different epitopes on the 21 hexon types among them a genus and three type specific ones and 18 different bi- and multilateral IT specific epitopes that grouped adenoviruses within the genus, independently from the subgenus they belong to.

The distribution of the distinct IT specific epitopes on the different hexon types has been different and varied between 1 and 17. By pairwise analysis ten human hexon types have formed three epitope clusters showing identical epitope spectra, namely type 4 and 19 (cluster I), types 8, 9, 9/13 and 10 (cluster II), as well as types 1, 2, 5 and 6 (cluster III) containing 17, 16 and 14 identical epitopes, respectively and no different epitopes at all. Close antigenic relationship was demonstrated between the types of the three different epitope clusters, each two given types containing 13 to 16 identical and only 1 to 4 different IT specific epitopes. HAdV types 7, 3, 18, 26 and SAdV type 16 also display a closer or looser but significant antigenic relationship among each other and to the members of the three clusters. HAdV serotypes 12, 27, 35, 41 and bovine adenovirus types 2 and 3, however, have shown a loose antigenic relationship with at least two or more other adenovirus types. Adenovirus type-pairs having the loosest antigenic relationships are shown on Table I. The number of identical epitopes on the given two hexon types varies between 0 to 6, and that of different epitopes on any given type-pairs are nearly double or manifold than that of the identical epitopes. Each two HAdV and/or BAdV serotypes determined by the meeting points are probably the best alternates for sequential use in experimental gene therapy.

On the basis of the present data a total of 31 different adenovirus type-pairs or small groups of types could be chosen for preparation of second or multiple recombinant vectors. For example in case of HAdV type 5, which is the most frequently used vector worldwide in the experimental gene therapy, for the second or multiple administration a recombinant vector could be used prepared from (i) bovine adenovirus type 3 which has no identical IT specific epitope with type 5, although the number of the different epitopes on the two types is 15, (ii) human adenovirus type 35

which has 5 identical epitopes, but the two types contain 13 different epitopes, (iii) bovine adenovirus type 2 having 5 identical epitopes and the two types contain 11 different epitopes, (iiii) human adenovirus type 12 having 6 identical epitopes with type 5, and the two types contain 11 different epitopes on their hexons.

Immune response of the host is induced not only by the genus and the type specific but also by the several IT specific epitopes, which have been found in different numbers and combinations on the different adenovirus serotypes. If well-chosen different serotypes are used as vectors for the first and second multiple administration the harmful effect of antibodies induced by the numerous IT specific epitopes could be eliminated. On the base of these data authors suggest the use of multiple vectors in gene therapy, each prepared from different adenovirus serotypes. The vectors would be used sequentially when second or multiple administration is needed.

Acknowledgements. The research program was supported by the grant No. 017052 and 23619 of the Hungarian Research Fund (OTKA) and by the Hungarian Academy of Sciences (TKI).

References

1. Chirmule,N., Propert,K.J., Magosin,S.A., Qian,Y., Qian,R., Wilson,J.M.: Immune response to adenovirus and adeno-associated virus in humans. Gene Ther **6,** 1574 (1999).
2. Gropp,R., Frye,M., Wagner,T.O.F., Bargon,J.: Epithelial defensins impair adenoviral infection: implication for adenovirus-mediated gene therapy. Hum Gene Ther **10,** 957 (1999).
3. Ji.,L., Bouvet,M., Price,R.E., Roth,J.A., Fang,B.: Reduced toxicity, attenuated immunogenicity and efficient mediation of human p53 gene expression in vivo by an adenovirus vector with deleted E1-E3 and inactivated E4 by GAL4-TATA promoter replacement. Gene Ther **6**, 393 (1999).
4. Lee,J.H., Zabner,J., Welsh,M.J.: Delivery of an adenovirus vector in a calcium phosphate coprecipitate enhances the therapeutic index of gene transfer to airway epithelia. Hum Gene Ther **10**, 603 (1999).
5. Ádám,É., Nász,I., Lengyel,A.: Characterization of adenovirus hexons by their epitope composition. Arch Virol **141,** 1891 (1996).
6. Ádám,É., Nász,I., Hudecz,F., Lengyel,A., Mező,G., Dobay,O.: Characterization of intertype specific epitopes on adenovirus hexons. Arch Virol **143,** 1669 (1998).