Table 5. Experiment IV: Serotype-specifc detection of the vector genomes in the various tissues. Monkeys injected with the mixture of AAV2(EGFPtub), AAV2/10(EGFPatub) and AAV2/11(EGFPbtub) (1.0×10^{10} gc each) were sacrificed at 5 or 7 months after the injection

Tissue	Monkey								
	#15 (3m)			#16 (3m)			#17 (3m)		
	2	2/10	2/11	2	2/10	2/11	2	2/10	2/11
Cerebrum	−	−	−	−	−	−	−	−	−
Cerebellum	−	−	−	−	−	−	−	−	−
Bone marrow	−	−	−	−	−	−	−	−	−
Skin	−	−	−	−	−	−	−	+	−
Retina	−	−	−	−	−	−	+	−	−
Muscle	−	−	−	−	−	−	−	−	−
Trachea	−	−	−	−	−	−	−	−	−
Lung	−	−	−	−	−	−	−	−	−
Heart	−	−	−	−	−	−	−	−	−
Liver	+	+	−	−	−	−	−	−	−
Gallbladder	−	−	−	−	−	−	−	−	−
Pancreas	−	−	−	−	−	−	−	−	−
Spleen	++	++	++++	++	++++	−	++	+++	++
Esophagus	−	−	−	−	−	−	−	−	−
Stomach	−	−	−	−	−	−	−	−	−
Jejunum	−	−	−	−	−	−	−	−	−
Ileum	−	−	−	+	+	−	−	−	−
Colon	+	−	−	+	+	−	+	−	−
Kidney	−	−	−	−	−	−	−	−	−
Adrenal gland	−	−	−	−	−	−	−	−	−
Bladder	++	++	+++	−	−	−	−	−	−
Tonsil	+	++	++	+	++	−	−	−	−
Thymus	−	−	−	−	−	−	−	−	−
Parotid gland	−	−	−	−	+	−	−	+	−
Submandibular gland	−	−	−	+	+++	−	−	−	−
Thyroid gland	−	−	−	−	−	−	−	−	−
Axillary lymph node	+	++	++	+	+++	−	+	++	+
Hilar lymph node	−	++	+	+	++	−	+	++	−
Mesenteric lymph node	+	+	+	−	+	−	++	++	−
Iliac lymph node	−	−	−	+	++	−	++	++	+
Inguinal lymph node	+	++	++	+	++	−	−	+	−
Testis/Ovary	−	−	−	−	−	−	−	−	−
Epididymis/Uterus	−	−	−	−	−	−	−	−	−

(−), $<10^2$ gc/0.5 μgDNA; (+), 10^2-10^3 gc/0.5 μgDNA; (++), 10^3-10^4 gc/0.5 μgDNA; (+++), 10^4-10^5 gc/0.5 μgDNA; (++++), $>10^5$ gc/0.5 μgDNA.

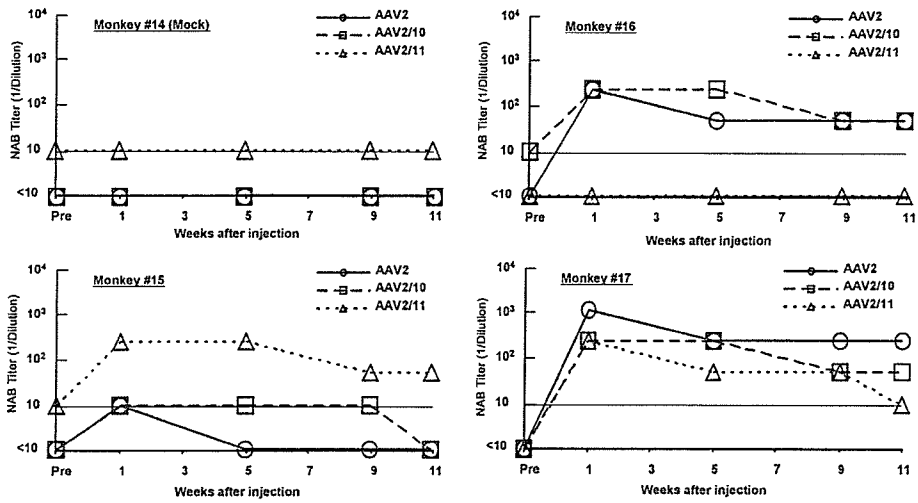

Fig. 4. Induction of anti-AAV neutralizing antibodies in monkeys. The serum from the monkey injected with saline (monkey #14) and the sera from those infected with the mixture of AAV2(EGFPtub), AAV2/10(EGFPatub), and AAV2/11(EGFPbtub) (monkeys #15, 16, and 17) were examined for their neutralizing activities by inhibition of transduction of COS-1 cells by the AAV vectors expressing beta-gal. Neutralizing titer (NAB Titer) of the antiserum was expressed as the reciprocal of the highest dilution that repressed the number of beta-gal positive cells to half of the number obtained with the samples mixed with the similarly diluted serum from a non-immunized mouse.

liver, gallbladder, pancreas, colon, ovary, uterus, and other organs (Table 2). The transfer of AAV2 vector to the brain via the blood-brain barrier agreed with the results of previous studies; when AAV2 vector has been injected into the liver or lung of non-human primates, vector DNA has been detected in the brain (6,8). No histological abnormalities or inflammatory reactions were observed in the tissues positive for the vector DNA. These results indicated that the vector in the blood was not readily excreted in the urine and rapidly attached to (or trapped by) various tissues, most readily the lymphoid tissues, without inducing any clinical symptoms. The presence of the vector DNA in the lymphoid tissues was consistent with previous findings; when an AAV2 vector containing the EGFP gene was injected into the liver of rhesus monkey fetuses, 0.01 - 0.05% of the monocytes in the spleen and lymph nodes of the infants were EGFP-positive (8). However, such EGFP-positive cells have not yet been characterized in detail.

Although the distribution patterns of the AAV2 vector at 3, 5, and 7 months pi varied from monkey to monkey (Tables 3, 4, and 5), the vector DNA was detected in various tissues of all of the monkeys sacrificed in this study. There was a tendency that from 2 days to 3 months pi, the vector DNA level was lowered to 1/100 in the spleen and to 1/2 in the other tissues. From 3 months pi to 5 months pi, the decrease in the vector DNA in the tissues, including that in the spleen, was marginal. The data strongly suggest that the AAV2 vector DNA would be maintained in various tissues for a long period of time, probably for years, without inducing any clinical symptoms.

The expression of the transgene was observed, at least in the lymph nodes, at 3 months pi. Although this remains to be determined, it is possible that the transgene could be expressed in other tissues harboring the vector DNA at a lower level. Then, the safety of the in vivo administration of AAV vectors would be affected by the properties of the transgene product.

AAV2/10(EGFPatub), AAV2/11(EGFPbtub), and AAV2 (EGFPtub) showed a similar pattern of vector distribution throughout the monkey tissues (Table 5). Previously, AAV2/10 and 2/11 vector DNAs were detected in the muscle tissues of BALB/c mice injected with AAV2/10 and 2/11 vectors (2×10^{11} gc/kg weight) via the tail vein at 6 weeks pi, which suggested that these vectors preferentially enter muscle cells (12). However, no AAV2/10(EGFPatub) and AAV2/11 (EGFPbtub) vector DNAs were detected in the muscle tissues of monkeys #15, 16, and 17 (Table 5). This apparent discrepancy may be ascribed to the difficulty of detecting vector DNA in the muscles of monkeys administered with a low vector dose (2×10^9 gc/kg weight) such as that used in this study.

The vector distribution obtained in this study is consistent with those reported in previous studies, in which AAV vectors have been instilled in the bronchial epithelium of rhesus monkeys (6) and have been injected into the liver of rhesus fetuses (8) and injected into the muscle of rhesus and cynomolgus monkeys (7). That is, AAV vectors that enter the circulating bloodstream appear to be distributed to various tissues, primarily the lymphoid tissues, and are maintained for a long period of time. Since a small portion of the tissues was subjected to examination for the presence of vector DNA and transgene products, it cannot be ruled out that the vector genome and the transgene products were present in other portions of tissues. Furthermore, extensive histological studies of the tissues and organs will be necessary in order to define the vector DNA-positive cell-species (e.g., liver cells, lung cells, or lymphocytes) and to accurately identify those cells targeted by the vector. In addition, the long-term consequences of vector persistence remain unknown.

The distribution and persistence of the AAV vectors varied from monkey to monkey. This variation is thought to be associated, at least in part, with the genetic heterogeneity of the monkeys. More data with other monkeys should be gathered in order to evaluate safety issues related to gene therapy using AAV vectors.

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