MOLECULAR ANALYSIS OF ADENOVIRUS ISOLATES FROM PREVIOUSLY VACCINATED YOUNG ADULTS

D. A. Blasiole
D. Metzgar
L. T. Daum
M. A. K. Ryan
J. Wu
C. Wills
C. T. Le
N. E. Freed
C. Hansen
G. C. Gray
K. L. Russell

Report No. 03-22

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Molecular Analysis of Adenovirus Isolates from Vaccinated and Unvaccinated Young Adults

Daniel A. Blaslie,¹ David Metzgar,² Luke T. Daum,² Margaret A. K. Ryan,¹ Jangvo Wu,¹ Christopher Wills,¹ Charles T. Le,¹ Nikki E. Freed,¹ Christian J. Hansen,¹ Gregory C. Gray,³ and Kevin L. Rust¹

¹Department of Defense Centers for Disease Control and Prevention, Naval Health Research Center, San Diego, California 92186-5122; ²Brooks City Base, San Antonio, Texas 78235; and Division of Biological Sciences, University of California, San Diego, La Jolla, California 92037

Received 25 September 2003; Returned for modification 10 October 2003; Accepted 25 November 2003

Infections of adenoviruses type 4 (Ad4) and Ad7 were discovered among previously vaccinated individuals through laboratory respiratory illness surveillance at military recruit camps. Genetic analysis was performed on these isolates and a sample of adenovirus isolates from unvaccinated patients. Antigenic regions of the adenovirus hexon gene from 21 vaccinated and 31 unvaccinated patients were sequenced and compared to homologous regions of Ad4 and Ad7 vaccine strains and of other representative hexon sequences archived in GenBank. The phylogenetic distribution of sequences from vaccinated individuals closely resembled those from unvaccinated individuals. The most common Ad7 strain was the Ad7A1 hexon genotype, and the most common Ad4 strain was a genotype nearly identical to the recently discovered ZG-95-873 Ad4 variant. Near exclusive isolation of Ad4 since 1999 indicates that the Ad4 variant is currently responsible for the vast majority of adenovirus morbidity in military recruit camps. Differences of nonsynonymous to synonymous nucleotide substitution rates in known antigenic regions compared to nonantigenic regions indicated positive selection for diversity in the antigenic regions and purifying selection in the nonantigenic regions.

Adenovirus was first discovered in 1953 (12, 22). Today, adenoviruses are classified into species (formerly subgenera) (A to F), based primarily on differences in their pathogenesis properties, and by their serotype, based upon neutralization with type-specific animal antisera (17). There are currently 51 recognized serotypes of human adenovirus (5). The classical method for subtyping adenoviral isolates is whole-genome digestion via a stepwise, systematic restriction enzyme analysis (REA) process. Restriction enzyme classification methods use a numbering and lettering system appended to the serotype number to distinguish unique strains (16). The letters "a" through "k" represent restriction enzyme whole-genome electrophoretic banding patterns by the reagent enzyme BamHI. An Arabic numeral is added when additional enzymes are used to further distinguish whole genomes. Intuitively studied in the 1950s and 1960s, adenoviruses were found to infect up to 80% of military recruits and lead to hospitalization in up to 20% (9). Adenovirus type 4 (Ad4) and Ad7 were the primary serotypes responsible for this morbidity and together constituted 60% of all hospitalized cases of acute respiratory disease (ARD) among military recruits (9). Live, enteric-coated oral vaccines, which induce immune responses through selective infection of the gastrointestinal tract, were developed first for Ad4 and later for Ad7 (13, 28). These vaccines were shown to be safe and highly effective in the immunization of military trainees (28), and routine administration to recruits began in 1971 (10). The Ad4 and Ad7 vaccines together lowered ARD morbidity by 95 to 99% and total ARD morbidity by 50 to 60% during the period of vaccine use (10). However, the production of the vaccines was discontinued in 1996, and the remaining lots were retained until supplies were exhausted in early 1999 (10). Recently, the U.S. Department of Defense awarded a contract to Batt Laboratories, Inc., for resumption production of the vaccines. Population-based laboratory respiratory illness surveillance was initiated by the Naval Health Research Center in 1996 to document the epidemiology of adenoviruses during and after the period of vaccine loss (10). The surveillance program was originally established at four recruit training camps in the United States to define the burden of adenoviruses (10) but was later expanded to eight sites and included testing for other viral agents (24). This surveillance documented large increases in adenovirus morbidity and several fatal cases after the vaccine was discontinued (2, 10, 25), suggesting that the initial vaccine was efficacious for the majority of circulating pathogenic strains. However, several cases of Ad4 and Ad7 infection were discovered among previously vaccinated individuals, raising the possibility that newly emergent strains of adenoviruses had appeared. Recent research on the evolution of circulating adenoviruses has engendered concern about the efficacy of the old vaccine against current strains. In order to determine the suitability of the original vaccine strains for a new vaccine, a study of strain variation among the circulating Ad4 and Ad7 serotypes was conducted in 1999 (5). The antigenic regions of the hexon gene from prototype, vaccine, community-acquired, and military wild-type strains collected from 1953 to 1997 were sequenced
and compared. Whereas the hecnon antigens of AD7 were
generally conserved over time, an AD7 variant (strain ZG95-877)
with nine amino acid changes in the hecnon antigen was found
to have been circulating since 1995 (5). These changes were
noted to confer decreased neutralization. The abilities of this
strain to cause infection among vaccinated individuals, how-
ever, was not investigated. Another recent study analyzed AD7
isolates from the United States from the years 1960 to 2000 by
whole-genome REA (8). The study noted the appearance of
two genome types previously undocumented in North Amer-
ica, AD7/d and AD7/h, which indicated a shift in the predomi-
nant AD7 genotype circulating in the United States. The hecnon
protein of AD7/d2 contains only one unspliced amino acid substi-
tution, but it has possible antigenic implications.

The primary objective of the present study was to determine
whether the adenoviruses infections among previously vacci-
nated individuals who had been between 1960 and 2000 and
resulted from prototype strains, currently discovered adenovirus
variants, or a completely new variant. A 1,200-bp region of the
adenovirus capsid protein gene contains seven discrete hypersensitive regions (HVR) to HVR that account for
>99% of the type-specific variation and code for the type-
specific epitopes on the protein (4, 5). This region of the hecnon
gene from adenovirus isolated from vaccinated individuals and
unvaccinated individuals was sequenced and compared to the
vaccine strains, the AD7 variant strain, and the other exist-
ing AD7 and AD7 hecnon sequences in GenBank. The results of
the present study will help guide future vaccine development
initiatives.

MATERIALS AND METHODS

Nasal Health Research Center survival for feline respiratory illness. The
Nasal Health Research Center has conducted population-based surveillance for
feline respiratory illness using basic training techniques since 1995. Presently
include Fort Lewis (South Carolina), Fort Lomard (Missouri), Great
Pyrenees National Reserve Training Command (Hispanic), Marine Corps Recruit Depot
(California), Fort Drum (Georgia), Joint Air Force Base (Florida), Fort
Irwin (California), and Camp May (New Mexico). Weekly rates of feline
respiratory illness, defined by an oral temperature of >39°C and either a cough
or wheeze, are reported. Records meeting the case definition are used to
permit the collection of a throat swab specimen and to assign a feline
species. Specimens are stored in ambient transport medium (Remel, Lenexa, Kansas)
and sent to the Nasal Health Research Center Respiratory Disease Laboratory, San Diego, California, for analysis. Classic viral
culture and isolation are performed using the MLA cell line (Virology, Milwaukee, Wisconsin) and BHK-21 (Virology, Camden, Arkansas) cell lines. Collected estimations of viral growth are pro-
duced for identification of the viral pathogens by using immunofluorescence.
A mixture of selected infectious strains for adenoviruses are further
analyzed by immunostaining using type-specific antisera to determine type and
subtypes previously described (6).

Viral isolation. Adenovirus-positive samples, negative for other respi-
atory pathogens, were considered for inclusion in the study. In this
instance, adenovirus was likely the causative agent of illness in the symptomatic cat at the time of the collection. The vaccination status of the host (vaccinated or un-
vaccinated) and sex (shih tzu or AD7), as previously determined by molecularis-
ation, were then considered. Vaccinated individuals were defined as those with feline
respiratory illness beginning >10 days after documented receipt of the
AD7 and AD7 vaccine. The AD7 sample set consisted of 10 isolates from vac-
vaccinated individuals and 30 from unvaccinated individuals and represented six
of the eight research camps participating in surveillance. The AD7 sample set con-
sisted of 11 isolates from vaccinated individuals and 1 from an unvaccinated individual. A low incidence of AD7 in recent years, with the exception of an
outbreak at Great Lakes in the Fall of 1997 (23), limited the availability of clinical isolates from unvaccinated individuals for this set. However, additional

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<th>DNA Sequence</th>
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<tr>
<td>AD7/d</td>
<td>5'-ATTCAATGACAAGGCTTAGC-3'</td>
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<td>AD7/h</td>
<td>5'-CAACATCCTTGTTGAGGCG-3'</td>
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<td>AD7/7B</td>
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<td></td>
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<tr>
<td>AD7/B1</td>
<td>5'-GGTGCCTTGGAGGAGGCG-3'</td>
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TABLE I. Adenovirus hecnon and fiber geneprimers

PCR amplification and sequencing. All selected samples were grown in
40% calf serum from original patient specimens. Viral DNA was extracted with the
QIAamp DNA Blood Mini Kit (Qiagen, Valencia, California). The early conserved
genome region and subregion-defining hypersensitive regions of the adenovirus hecnon,
corresponding approximately to the first 1,300 bp of the gene, was amplified by
PCR with the primer UP and SL, as previously described (5). The reaction
mixes contained 1× PCR Buffer III (Applied Biosystems, Foster City, Califor-
nia) 2.5 mM MgCl2, 200 μM concentrations of each dNTP (Applied Biosys-
tems), and 1 μl of DNA template in a total volume of 50 μl. The cycling
consisted of an initial denaturation at 94°C for 2 min, followed by 30 cycles of
30 seconds for 4°C, 30 seconds for 5°C, and 30 seconds for 3°C, with a final extension of
1 min for 7°C. The amplicons were purified with QIAamp PCR purification kit
(Qiagen).

Purified adenovirus hecnon sequences were cycle sequenced with the Perkin-
Elmer ABI 377 thermocycler (PE Biosystems, Foster City, Califor-
nia), using the Big Dye terminator cycle sequencing Kit (PE Biosystems). The sequencing
consisted of 25 cycles of 4°C for 30 s, 5°C for 20 s, and 4°C for 4 min. All
products were sized by using GeneScan-l00 LIZ (Applied Biosystems). The primers used for sequencing included two PCR primers (UP and SL),
three internal primers (B2, B1, and B1) (Table 1). Cycling parameters were mostly
consistent with those previously described (5) and four novel internal primers (B2C, B2D, and B1) (Table 1). Curing parameters were mostly
consistent with those previously described (5) and four novel internal primers (B2C, B2D, and B1) (Table 1). Curing parameters were mostly
consistent with those previously described (5) and four novel internal primers (B2C, B2D, and B1) (Table 1). Curing parameters were mostly
consistent with those previously described (5) and four novel internal primers (B2C, B2D, and B1) (Table 1).
RESULTS

Surveillance serotype distribution. The proportional distribution of adenovirus serotypes isolated from military recruit camps from 1996 to 2002 differed markedly (Fig. 1). In 1996, during the last year of routine vaccine administration, Ad21 constituted the majority (58%, 14 of 24) of adenovirus morbidity, whereas Ad4 and Ad7 constituted only 4% each. Upon tapered use of the vaccine in 1997, however, Ad4 and Ad7 morbidity rose to 58% (939 of 1,618) and 26% (427 of 1,618), respectively, with other adenovirus serotypes constituting the remaining 6%. In 1998, Ad7 virtually disappeared, whereas Ad4 increased to 73% (191 of 260). The vaccine was completely depleted in 1999, with Ad4 responsible for 98% (247 of 248) of adenovirus morbidity. Ad4 remained at least at this proportion for the remainder of the analysis period with negligible representation from other serotypes.

Phylogeny and mutational characteristics. Phylogenetic analysis revealed a branching of the Ad7 sequences into two predominant groups. One group (Fig. 2, prototype group) contained the three known prototype sequences (only AF169065 represented on the tree) and one military sample (AF169066). The other group (Fig. 2, vaccine-strain group) contained the Ad7a vaccine strain, S-1058 Ad7a prototype, Ad7d prototype, one sequence from an unvaccinated military patient (AF169067), 10 sequences from vaccinated military patients, and three other sequences from Geoduck.

The Ad7 vaccine-strain group was distinguished from the prototype group by 25 coding differences (Fig. 2a). The vaccine strain was identical to only one military strain (AF169065), which was from a vaccinated individual. The other 10 military isolate sequences in this group differed by at least one amino acid. Sequences of these strains had L443Q (HVR1) substitution, which was shared with the Ad7d2 strain and the prototype group (Fig. 2a). Of the 11 samples from vaccinated military patients, 7 carried L443Q.

Ad4 also divided into two main phylogenetic lineages. The first lineage (Fig. 2, vaccine-strain group) contained the Ad4 vaccine strain, the B1-67 prototype, and three military samples from 1998 and earlier (62-G196.V, 62-F196.V, and 39-G197.V). The second group (Fig. 2, variant group) contained the Z-G 95-873 variant, an isolate from Korea (AF154012), and the remaining 33 military isolates. All 3 military sample sequences in the vaccine-strain group were from vaccinated individuals compared to 4 of 33 in the variant group. The variant strain comprised 91.7% (33 of 36; 95% confidence interval, 78.2 to 97.1%) of all Ad4 samples sequenced since 1996 and 100% (16 of 16; 95% confidence interval, 80.0 to 100%) since 1999.

The variant group was distinguished from the Ad4 vaccine-strain group by 10 amino acid substitutions (Fig. 3b). Of the 33 military samples in the variant group, 22 had identical amino acid sequences (Fig. 3b, sequence AY133724). Two exceptions (40-FW97.V and S1-LACK00.NV) contained a nonconservative K218N substitution, as defined by differences in size and charge (4), which they shared only with the Z-G 95-873 variant and the vaccine strain (Fig. 3b).

Synonymous versus non-synonymous substitutions. Pairwise comparison of conserved regions within each serotype yielded significantly greater mean synonymous substitution rates than mean non-synonymous substitution rates (Table 2). In contrast, pairwise comparison of hypervariable regions within each serotype yielded greater mean non-synonymous substitution rates than mean synonymous substitution rates. The differences between the means in the hypervariable regions were not significant; however, 103 of 231 independent pairwise comparisons among the Ad7 sequences had significantly greater non-synonymous substitution rates, whereas only 10 had significantly greater synonymous substitution rates. Likewise, with the Ad4 sequences 319 of 780 comparisons had significantly greater non-synonymous substitution rates, and 26 had significantly greater synonymous substitution rates. The large number of nonsignificant pairwise comparisons was primarily the result of a high level of sequence redundancy in our data set.

Atypical strains. Four samples (three vaccinated and one unvaccinated), all isolated in 1998 and initially sequenced as Ad4, yielded mostly unscorable nucleotide chromatograms.
FIG. 2. Ad4 and Ad7 hexon maximum-likelihood protein tree with canine Ad1 root. Isolates sequenced in the present study are labeled according to the following format: last two digits of accession number of identical sequence, location of isolation, year of isolation, canine, state, and strain. FB, Fort Benning, PJ, Fort Jackson; FLW, Fort Leonard Wood; LACK, Lackland; GL, Great Lakes; MCRD, Marine Corps Recruit Depot; V, vaccinated; UV, unvaccinated. Full accession numbers appear once in parentheses. Accession numbers in boldface correspond to those in Fig. 3. Multiple accession numbers among identical protein sequences are due to DVA sequences with silent mutations. Bootstrap values greater than 50 (of 100 total bootstraps) are shown. Rescaled consistency index = 0.97.
but short portions of readable fragments suggested the strains were Ad5. Primers Ad5SH and Ad5HR were designed from the clean exon segments, and primers Ad5FIB-SF and Ad5FIB-
SH (Table 2) were designed from existing Ad5 sequences in GenBank. Both primer sets were used in sequencing, and the resulting sequences showed 100% identity (288 of 288 and 274 of 274, respectively) with Ad5 in GenBank (versions 2.2.5 and 2.2.6). Two further confirmatory tests included a type-specific PCR assay (19) and a second microneutralization analysis, both of which verified the Ad5 diagnoses and showed no evidence of Ad4 coinfection.

**DISCUSSION**

Infection with mutant adenovirus strains constitutes one of the many possible reasons for vaccine failure. Improper storage or administration of vaccine, asymmetric carriage of adenovirus that is not the cause of acute illness, or imperfect vaccine efficacy are all viable explanations for the isolation of adenoviruses among vaccinated individuals. With the development of a new adenovirus vaccine under way, however, it is vital to address the hypothesis of strain variation with these uniquely available isolates. Although it may be impossible to

**TABLE 2. Mean synonymous and nonsynonymous substitution rates in conserved and hypervariable regions of Ad7 and Ad4**

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<th>Serotype</th>
<th>Conserved regions</th>
<th>Hypervariable regions</th>
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<td></td>
<td>Mean substitution size ± SD</td>
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<tr>
<td>Ad7</td>
<td>39.9 ± 6.4</td>
<td>206 ± 8.8</td>
</tr>
<tr>
<td>Ad4</td>
<td>17.6 ± 3.6</td>
<td>10.2 ± 2.5</td>
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*Rates were determined through pairwise sequence comparison and are expressed as synonymous substitutions per 1,000 synonymous sites and nonsynonymous substitutions per 1,000 nonsynonymous sites.*
determine whether one mutation or set of mutations was re-
sponsible for infection, consideration of these changes among
circulating viral strains is an important step.

The major phylogenetic bifurcation within Ad7 hexon se-
quences was previously shown (5, 8, 14) and provided genetic
evidence for a distinction originally seen only at a phenotypic
level by cross-neutralization and REA (5). An eightfold reduc-
tion in neutralization of the Goren Ad7 prototype compared to
the S-160S Ad7a strain with Ad7a antiserum was also pre-
viously shown (5). This neutralizing difference may have con-
tributed to a breakdown of the sole strain from a vaccinated
patient in the prototype group (58.8L.90-V; Fig. 2), but the
low number of prototype-like strains isolated in the present
study indicated that they are not currently a major epidemi-
ological threat in recruit camps.

A recent REA study on the 1997 Great Lakes outbreak
showed >70% of the samples were of the Ad7d2 genome type
(8). It was also found in the same study, as elsewhere (14), that
an L430Q hexon substitution in HV7R is consistently linked
with Ad7d and Ad7d2, as distinct from Ad7a, Ad7b, Ad7c,
Ad7g, and Ad7h. Of 10 samples in our study from the same
outbreak but from vaccinated individuals, 7 also contained the
L430Q substitution, indicating that these samples are most
likely of the Ad7d2 genome type. The substitution is noncon-
servative and dramatically affects the hydrophilic—and prob-
able structure—characteristics of the protein by transforming
the nine-residue surrounding region in HV7R from a second-
ary-structure conformation that exposes primarily hydrophobic
amino acid residues to a conformation that exposes primarily
hydrophilic residues (14). It has therefore been suggested that
this substitution imparts antigenic implications (8), which may
explain the high number of Ad7d of this type isolated from
vaccinated individuals in our study. In contrast, however, evi-
dence showing a rapid decrease in AV-associated illness after
introduction of the vaccine in this outbreak (25) strongly sug-
gests that the strain is at least partially susceptible to vaccine-
induced immunity. Cross-neutralization data between Ad7a
and a strain containing the L430Q substitution would provide
more evidence of the antigenic implications.

Several other substitutions occurring among the Ad7s may
have contributed to infection in vaccinated individuals. The
nonconservative S423Y substitution in samples 56.9L.97.V
and 57.9L.97.V (Fig. 3a) resided in HV7R and may have
directly altered the epitope. In addition, isolated nonconser-
vative substitutions in nearly conserved regions may have in-
directly affected the antigens. The known epitopes are known
to be conformational, since only the protein in its native tertiary
form and not linear hexon peptides or heat-denatured mono-
meric proteins neutralize in vitro (4). Therefore, a change in a
structural region, such as the F0080 substitution (Figs. 3a,
55.9L.97.V) in the conserved region at the base of the loop
containing HV7R (1, 4), for example, may drastically affect
protein folding in the antigenic regions.

Given the predominance of Ad4 among cases of respiratory
illness in recent years, as well as the predominance of the
variant genotype within Ad4 (Fig. 1), it appears that this single
strain was responsible for nearly all adenovirus-associated re-
spiratory illness in military recruit camps since 1989. The con-
tinued increase in the dominance of this strain after vaccine
cessation suggests that its proliferation was not primarily a
result of vaccine use. It is difficult to say how well the prior
vaccine strain will clinically protect against this variant, since
the majority of the variant isolates were isolated after vaccine
use was drastically reduced. However, it was recently shown in
an in vitro cross-neutralization analysis that the ZG 95-873
variant has a forbiddingly reduced neutralization in compari-
son with the Ad4 RV-67 prototype strain (5), a strain similar in
genotype to one vaccine strain. The majority (32 of 35) of
genotypes in the variant group differed from the ZG 95-873
variant, as well as the vaccine strain, the N236k amino acid
substitution (Fig. 3b; sequence ATY32247). If this substitution
changes the epitope at all, it would render it further unrecon-
izable by vaccine-induced antibodies; however, it is unknown
whether this substitution would have such an effect, given its
ancestral character in a conserved region. Nevertheless,
due to the current prevalence of the Ad4 variant with the
N236k mutation, any new vaccine should be optimized for
effectiveness against this strain.

With Ad7, several isolated substitutions in the Ad4 set
have the potential to alter the epitope. Three nonconservative
amino acid changes (Fig. 3b; P540F in sample 39.9L.97.V,
N246k, and S423F) resided in antigenic regions. Nonidentical
nucleotide mutations code for the N246k substitution, which
suggests convergent evolution at this site, perhaps driven by
selection. However, given that only one sample from a vacci-
nated individual was found with each mutation, our data do
not conclusively show their clinical effects.

Nucleotide sites are classified as either non synonymous or
synonymous depending on whether or not, respectively, a mu-
lation at the site will lead to an amino acid substitution. The
first and second nucleotide sites in a codon are largely non-
synonymous, and the third site, due to redundancy in the ge-
netic code, is largely synonymous. The degree to which a site
is classified as either synonymous or non synonymous is deter-
mined by accounting for the coding implied by all possible
mutations. Within a gene region, the number of nonsynony-
ous substitutions per synonymous site relative to synonym-
yous substitutions per synonymous site indicates the type of
evolutionary selective pressure on the region. Equal rates of
nonsynonymous and synonymous mutations indicate neutral
drift, an excess of synonymous substitution rates indicates con-
servative evolutionary pressure (purifying selection), and an
excess of nonsynonymous substitution rates indicates selection
for diversity (diversifying selection). Evidence of diversifying
selection is often found in protein regions that benefit from
variation such as surface antigens of pathogens (7) and in
immunoglobulin variable regions (27). In our study, the non-
synonymous substitution rates in the antigenic (hypervariable)
regions of the sequences suggest that these regions are affected
by diversifying selection. In contrast, the nonantigenic (con-
served) regions show strong purifying selection, as is expected
from regions with a structural function. Given the propensity
for variation in the hypervariable regions, one would expect
that the selective pressure of vaccine etchings might eventu-
ally allow the virus to evolve resistance to vaccine-induced
immune responses. Antibiotic cross-neutralization analysis be-
tween vaccine strains and circulating strains should be closely
monitored.

A recent study of the adenovirus hexon (23) proposed a
reassignment of the hypervariable regions based on stretches

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of density in an alignment of 40 partial hexon amino acid sequences from both human and simian adenoviruses. The hypervariable regions as presented in the alignment in the study generally overlap with the assignments as previously defined for Ad4 and Ad7 (15). An analysis of mutation rates in the reasigned hypervariable regions of the sequences used in our study shows higher synonymous than nonsynonymous substitution rates in the hypervariable regions (data not shown), which is the po- pulation of what was found with the previous assignments. Ad- dey's support for the previous region assignments is pro- vided by a visual inspection of the alignment used to define the new assignments. This reveals that definite motifs in a subset of sequences, including that of Ad2, are shifted out of phase with those of Ad4, Ad7, and several others. Such a shift would affect identity scores assigned to each region and hence the place- ment of hypervariable regions in the alignment. Our data therefore support the hypervariable positions as previously defined for Ad4 and Ad7.

Initial use of the Ad2 vaccine in the 1960s was associated with Ad7 and Ad22 emerging as the chief etiological agents of ARD in military recruit camps. At the time, this constituted the first outbreak of Ad22 in the Western hemisphere (21). The isolation of three of the four Ad2s from vaccinated indi- viduals indicates that this serotype may have similarly filled an evolutionary niche left open during the period of Ad4 and Ad7 vaccine use, but to a much smaller extent. Recent evidence shows that Ad2 manifests a lower binding affinity and replicates more slowly in A549 cells relative to other adenoviruses in subgroups A, B (includes Ad7), D, and E (includes Ad11) (18). The A549-mediated growth typically used in the serological diagnosis of AV may fail to produce an Ad7 site sufficient for detection by microimmunofluorescence. The epidemiological impact of Ad2 on military recruit populations during the period of vaccine use may therefore have been underestimated. Molecular surveillance should be used to monitor for the possible emergence of Ad22, Ad7, or other serotypes upon reinstitution of the Ad4 and Ad7 vaccines.

Although the data in the study cannot definitively explain the reason for past vaccine failures, it shows the distribution of strains with possible partial resistance to the previous vaccines. Given the predominance of the Ad7 variant in recent years, an effective vaccine against it may significantly reduce adenovirus- induced respiratory infection in the military. Although Ad7 is not circulating at present, protection against the Ad42 strain would prevent any opportunistic proliferation upon con- trolling Ad4. Other serotypes have not shown Ad7's capacity to fill the Ad4 niche but, with decreasing selection acting on the hexon epitopes, continued surveillance for new emergent strains should be conducted.

ACKNOWLEDGMENTS

This report represents work performed during the U.S. Depart- ment of Defense Adenovirus Program (1975-1982) and the period 1982-1986.

The views expressed are those of the authors and do not reflect the official policy or position of the Department of the Army, the Department of the Navy, or the U.S. Government. This work was approved for public release (distribution unlimited). The research has been conducted in compliance with all applicable federal regulations governing the protection of human subjects in research under protocols 32367.

We thank Anthony W. Hankowsky of the Department of Defense Cytogen for Development Health Research for organization and analysis of clinical disease surveillance data. We also thank Lea K. Crawford- Myers and Donald P. Schriver of the Viral and Rickettsial Disease Laboratory, Division of Communicable Disease Control, California State Department of Health Services, for adenovirus antiserum and expert consultation.

REFERENCES

**REPORT DOCUMENTATION PAGE**

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<th>9. PERFORMING ORGANIZATION REPORT NUMBER</th>
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<tbody>
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| 10. Sponsor/Monitor's Acronyms(s) |

| 11. Sponsor/Monitor's Report Number(s) |

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<th>12. DISTRIBUTION/AVAILABILITY STATEMENT</th>
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<td>Approved for public release; distribution unlimited.</td>
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<th>13. SUPPLEMENTARY NOTES</th>
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<td>Published in: Journal of Clinical Microbiology, 2004, 42(4), 1686-93</td>
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<tr>
<th>14. ABSTRACT (maximum 200 words)</th>
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<tr>
<td>Infections of adenovirus type 4 (AV4) and type 7 (AV7) were discovered among previously vaccinated individuals through febrile respiratory illness surveillance at military recruit camps. Genetic analysis was performed on these isolates and a sample of adenovirus isolates from unvaccinated patients. Antigenic regions of the adenovirus hexon gene from 21 vaccinated and 31 unvaccinated patients were sequenced and compared with homologous regions of AV4 and AV7 vaccine strains and of other representative hexon sequences archived in GenBank. The phylogenetic distribution of sequences from vaccinated individuals closely resembled those from unvaccinated individuals. The most common AV7 strain was the AV7d2 hexon genotype, and the most common AV4 strain was a genotype nearly identical to the recently discovered Z-G 95-873 AV4 variant. Nearly exclusive isolation of AV4 since 1999 indicates that the AV4 variant is currently responsible for the vast majority of adenovirus morbidity in military recruit camps. Different ratios of nonsynonymous to synonymous nucleotide substitutions in known antigenic regions compared with nonantigenic regions indicated positive selection for diversity in the antigenic regions and purifying selection in the nonantigenic regions.</td>
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<td>adenovirus-4, adenovirus-7, hexon gene, respiratory illness, vaccines, sequencing, military recruits</td>
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<tr>
<td>a. REPORT</td>
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<tr>
<td>b. ABSTRACT</td>
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<td>c. THIS PAGE</td>
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<th>17. LIMITATION OF ABSTRACT</th>
<th>18. NUMBER OF PAGES</th>
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<tr>
<th>19a. NAME OF RESPONSIBLE PERSON</th>
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<td>Commanding Officer</td>
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<th>19b. TELEPHONE NUMBER (INCLUDING AREA CODE)</th>
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<td>COMM/DSN: (619) 553-8429</td>
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