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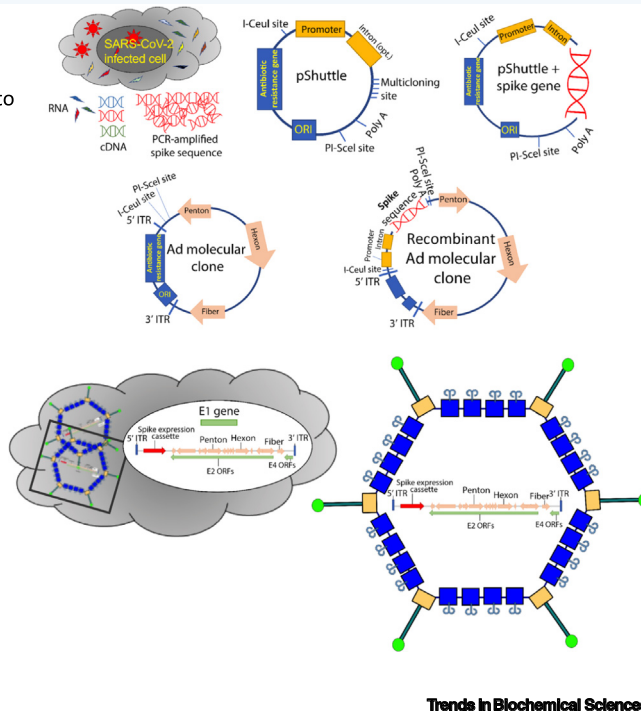
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# COVID-19 Vaccines Based on Adenovirus Vectors

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**Step 1:** Isolate RNA from SARS-CoV-2 infected cells, clone amplified spike sequence into pShuttle vector, clone expression cassette from pShuttle into the Ad molecular clone.



**Step 2:** Transfect E1 helper cell line with linearized Ad molecular clone. Amplify, purify, and quality control rescued Ad vector.

**Step 3:** Conduct preclinical animal experiments.

**Step 4:** Conduct clinical trials.

**Step 5:** Gain regulatory approval for use in humans.

**ADVANTAGES:**

Ad MCs for different human (AdHu) or chimpanzee Ad (ChAd) viruses are available, which allows for production of experimental spike vaccines within 3–4 weeks.

Procedures for large-scale GMP production and release testing have been developed.

Ad-spike vaccines were shown to be safe in humans.

Ad-spike vaccines induce potent and sustained T and B cell responses to the spike protein in young and aged individuals.

Ad-spike vaccines tested thus far have provided protection against coronavirus disease 2019 (COVID-19): Sputnik V, Gamaleya (AdHu26 prime/AdHu5 boost): 91.4%; AZD155, AstraZeneca (ChAdOx1, 2X): 62.1–90.0%, both vaccines completely protect against severe disease; Johnson & Johnson (AdHu26, 1X): 66%, 85% protection against severe disease.

Adenovirus (Ad) vectors are produced from molecular clones (MC) of the Ad genome. E1 and E3 domains are deleted; removal of E1 prevents virus replication. The genome is cloned into a plasmid vector. Infected cells provide viral RNA, for example, spike of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Spike sequences are amplified and cloned into a shuttle vector from where the expression cassette is excised and inserted into an Ad MC. Ad MC transfection of E1<sup>+</sup> helper cells rescues the vaccine, which is expanded, tested, and ready for good manufacturing practice (GMP) production and clinical trials.

Ad-spike vaccines can be based on different Ad serotypes, which allows for heterologous prime-boost immunizations, which are more effective than repeated use of the same Ad vector.

Ad-spike vaccines can be stored at 4°C.

Ad-spike vaccines are relatively inexpensive.

**CHALLENGES:**

Neutralizing antibodies to common human serotypes of Ad viruses reduce vaccine immunogenicity.

Neutralizing antibodies to the Ad vector induced by the first immunization reduce immune responses to a second immunization with the same Ad vector.

Antigen encoded by the Ad vaccine persists for a period of time, which delays transition of lymphocytes into memory, potentially requiring extended intervals between two vaccine doses.

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**Step 1:** Vaccine infects CAR<sup>+</sup> cells; this is inhibited by VNAs specific for the Ad vector. VNAs to other Ad viruses do not inhibit infection.

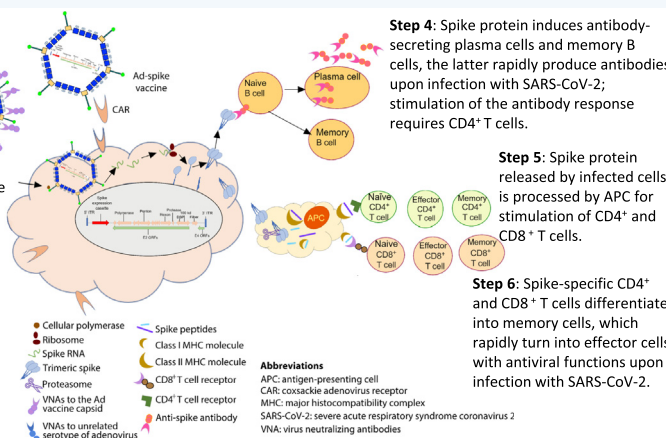
**Step 2:** In the infected cells, the Ad vaccine genome produces spike protein.

**Step 3:** Ad particles induce Ad-specific VNAs, which inhibit the effect of a second immunization with the vaccine.

**Step 4:** Spike protein induces antibody-secreting plasma cells and memory B cells, the latter rapidly produce antibodies upon infection with SARS-CoV-2; stimulation of the antibody response requires CD4<sup>+</sup> T cells.

**Step 5:** Spike protein released by infected cells is processed by APC for stimulation of CD4<sup>+</sup> and CD8<sup>+</sup> T cells.

**Step 6:** Spike-specific CD4<sup>+</sup> and CD8<sup>+</sup> T cells differentiate into memory cells, which rapidly turn into effector cells with antiviral functions upon infection with SARS-CoV-2.



Ad vaccines infect coxsackie adenovirus receptor (CAR<sup>+</sup>) cells. They produce spike protein, which induces antibody-secreting plasma cells and memory B cells. Antigen-presenting cells take up and process spike to bind to MHC antigens for stimulation of CD4<sup>+</sup> or CD8<sup>+</sup> T cells, which help activation of other cells or have antiviral functions. Ad vaccines induce virus neutralizing antibodies (VNAs) to Ad, which inhibit Ad vaccines; VNAs to different Ad serotypes have no effect.



## Declaration of Interests

No interests are declared.

## Literature

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