

between ~6000 and ~4000 years ago that is consistent with the marine dust-flux record (7). However, various simulations suggest that precipitation changed more gradually, accompanied by vegetation collapse in some models but steadier decline in others (8).

Even the continental geological evidence is

**Evolution of mid- to late-Holocene climate of North Africa.** Saharan dust flux over the Atlantic (4) increased sharply 5500 years ago [arrow in (A)], suggesting an abrupt end to the African Humid Period, whereas records from the West African Sahel (10) show a later rise [arrow in (B)]. A reduction in swamp forest in the Sahel (9), indicated by Guinean tree pollen (C), accompanied the demise of wetlands and broadly followed changes in insolation (D).

equivocal. The appearance of abrupt drying of the Sahara might have arisen from the removal of sediments from many basins by the wind following desiccation during the mid-Holocene. Added to this, there is a dearth of well-dated sites in the Sahara, truncated or otherwise (3). In the Sahel, the semiarid southern fringe of the present-day Sahara, continuous and well-dated lake-sediment sequences do not support the idea of abrupt drying either; here, changes in vegetation were gradual (9) (see the second figure, panel C) and dust flux increased later than 5500 years ago (see the second figure, panel B) (10). However, the extent to which these Sahelian records are more widely representative is uncertain.

The evidence from Lake Yoa reported by Kröpelin *et al.* adds a new dimension to the problem. The continuous and well-dated pollen record for this site shows no abrupt change in vegetation in the mid-Holocene. The rise in Lake Yoa's salinity was rapid, but this was almost certainly a response to a local threshold being crossed as the lake changed

from hydrologically open to hydrologically closed, rather than to abrupt climatic drying. The relatively smooth rise in dust flux is consistent with the gradual reduction in vegetation cover. Kröpelin *et al.* conclude that the vegetation feedbacks that have preoccupied modelers of the African monsoons must have been weaker than previously thought.

The record does not provide the last word on the tempo of Holocene aridification in North Africa, but it does raise important questions about Holocene environmental changes across the area and about the nature of feedbacks in the climate system. There is little point in calling for further continuous records to help resolve these issues: As Kröpelin *et al.* point out, suitable sites probably do not exist. However, improving existing geological records and using these to refine climate models would go a long way toward furthering our understanding.

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## AIDS/HIV

# A STEP into Darkness or Light?

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The outcome of the efficacy trial of an adenovirus serotype 5 (Ad5) vector-based HIV-1 vaccine last November (STEP trial) was unexpected. Not only was the vaccine ineffective at lowering plasma viremia postinfection, but it may have increased the risk of acquiring HIV-1 infection. Although firm conclusions cannot be drawn based on the small number of infections that occurred (49 in the vaccinated patient group, 33 in the placebo group), it has been suggested that vaccine-

induced generalized immune activation, which can promote HIV-1 replication, might have increased the infection risk (1).

The vector-based vaccine used in the STEP trial was a recombinant Ad5 virus expressing immunogenic HIV-1 proteins. A higher number of HIV-1 infections occurred in the subset of vaccinees with high, preexisting titers of Ad5-specific antibodies, compared with placebo recipients. One possible explanation is that anti-Ad5 antibodies facilitate cellular uptake of the Ad5 vector (perhaps by cells other than the ones normally targeted), inducing an immune response that enhances HIV-1 infection. Although immune responses to viral infections are usually protective, they can also be harmful (as with West Nile, dengue, measles, and respiratory syncytial virus infections). For example, a low-titer antibody re-

The recent failure of a vector-based HIV vaccine may be explained by individual subjects' immune capacity and genetics.

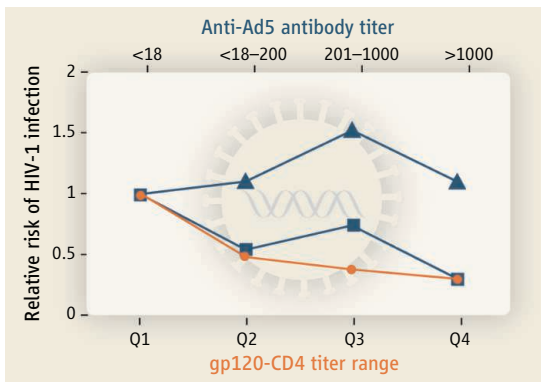
sponse to West Nile virus can enhance viral replication and exacerbate disease (2). Whether similar events occur after vaccination with an Ad5 or similar viral vector is now something to consider.

One way to examine the apparent effect of anti-Ad5 antibodies is to plot the relative risk of HIV-1 infection in the STEP trial groups as a function of antibody titer (see the figure). Unexpectedly, the higher the anti-Ad5 antibody titer in the placebo group, the lower the HIV-1 infection rate. By contrast, infection risk in the vaccinated group appears similar at high and low titers. Given statistical limitations, a conservative explanation is that these patterns arise by chance. However, because of the need to understand all aspects of the STEP trial, we here consider whether the data patterns are meaningful.

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It seems implausible that Ad5-specific antibodies directly protect against HIV-1 infection, as no reasonable mechanism is apparent. On the other hand, it may be that an individual with a high-titer Ad5-specific antibody response can better resist HIV-1 infection naturally. The anti-Ad5 antibody titer could thus be a surrogate marker for a host genetic constitution that confers reduced susceptibility to HIV-1. Other factors, perhaps including immune activation, might outweigh this effect in Ad5 vaccine recipients.

A possible precedent arises from two earlier HIV-1 vaccine trials with the gp120 glycoprotein protein of HIV-1 (AIDSVAX). The infection rates were almost the same in vaccine and placebo recipients, but the titers of gp120-binding antibodies and the risk of HIV-1 infection were inversely correlated (3) (see the figure). It



**Possible influence of antibody titers on risk of HIV-1 infection.** Data from (1) show the relative risk of HIV-1 infection in the Ad5-vaccinated (triangles) and placebo (squares) groups from the STEP trial, as a function of the anti-Ad5 antibody titer range. An anti-Ad5 antibody titer of <18 does not mean that an individual has never been exposed to Ad5, as some infected people have subthreshold responses. Data from (3) (circles) show the relative risk of HIV-1 infection in recipients of AIDSVAX (gp120) as a function of the titer range of antibodies that block gp120 from binding to CD4, a receptor used by HIV-1 to gain entry into host T cells (other measures of the antibody response to gp120 yield broadly similar plots). The confidence limits on both data sets (not shown) are broad. Both studies are large (5403 participants in the AIDSVAX trial; analysis of the STEP data is based on a subset of 1836 of those enrolled). The statistical significance of the trend shown for the AIDSVAX data is established (3), whereas significance of the trend among the STEP data is debatable (1). Comparison of the associations is therefore speculative. Not every individual may have been exposed to Ad5 in the STEP trial, whereas in the AIDSVAX trial, every vaccine recipient was given gp120.

is not likely that this correlation arose because binding antibodies are themselves protective. For the overall trial outcome to be neutral (the vaccine conferred no protection compared to the placebo), if above-average titers of gp120-binding antibodies directly protected against infection, then below-average antibody titers would also have to act directly, to place individuals at a greater risk of infection than placebo recipients. This seems improbable. The authors of this study argued that the ability to mount a strong anti-gp120 antibody response inversely measures susceptibility to HIV-1 infection. This scenario might also apply to high anti-Ad5 antibody titers in the STEP trial. Hence, individuals that can mount strong antibody and possibly other relevant immune responses to pathogen antigens may be inherently more resistant to HIV-1 infection—they have “better immune systems.”

There is a modest but significant tendency for individuals with weak antibody responses to one component of a vaccine against measles, mumps, and rubella (MMR) to also respond poorly to the other vaccines (4). Moreover, antibody responses to measles and mumps vaccines are influenced by host genetics, including

genes encoding antigen-presenting proteins [human leukocyte antigen (HLA)] that are important for immune function, and single-nucleotide polymorphisms in genes encoding cytokines or their receptors, such as interleukin-2 (IL-2), IL-10, and IL-12 receptor  $\beta$  (5, 6). An extrapolation to HIV-1 vaccines suggests that a broad range of host factors, not just those affecting humoral immunity, might influence protection. For example, IL-10 might affect vaccine responses (and susceptibility to infection) by influencing both cellular and humoral immunity (7, 8); gp120 triggers IL-10 production by specific immune cells (dendritic cells) to an extent that varies greatly between individuals (9), which may be relevant to understanding the wide range of anti-gp120 antibody titers seen in gp120 vaccinees (3).

Host genetic factors may confound HIV

vaccine trial evaluation (10–15). For example, genotypes of CCR5, the major HIV-1 co-receptor, and the gene copy number of CCL3L1, the most potent and HIV-1-suppressive CCR5 ligand, together influence cell-mediated immunity in both HIV-1-negative and -positive individuals (15). Genotypes associated with reduced cell-mediated immune responses were similar in the control and HIV-1-infected groups, and predicted an enhanced risk of acquiring HIV-1 and a faster disease course (15). Thus, an individual with a “better immune system” might indeed resist HIV-1 infection or partially control replication. This is consistent with observations that pre-seroconversion immune status predicts the rate of HIV-1 infection and rate of immune cell (CD4<sup>+</sup> T cell) depletion postinfection (15–17). A caveat against focusing narrowly upon the antibody response in the STEP trial is supported by the use of cell-mediated immunity-related parameters to define pre-seroconversion immune status in the aforementioned studies (15–17). Thus, risks for HIV-1 infection may be associated with risks for “a broader spectrum of immunological challenges” that are “reflected in the T cell repertoires of exposed individuals” (18).

More complexity is created when the same vaccine is tested in different geographic areas with genetically diverse populations. The STEP trial was conducted mostly in North America and the Caribbean, but a second, now abandoned, trial (PHAMBILI) was initiated in southern Africa. HIV-1–host interactions relevant to the natural history of the epidemic and vaccine responsiveness may be population specific. Indeed, CCR5 genetic determinants influencing AIDS progression rates differ in European Americans and African Americans (10). HIV-1 acquisition risk is a product of the susceptibility of uninfected persons and the communicability of HIV-1 from the infected person (19, 20). Communicability is dictated in part by the transmitter’s viral load (19–22), which is influenced by host genetics (14, 15), and also by the genetic makeup of both sexual

partners. For example, transmission is more efficient when sexual partners share similar HLA class I alleles (23). Thus, even small differences in the frequencies of disadvantageous genetic variants in different vaccine trial cohorts might have a disproportionately large effect on the likelihood that genetically “at-risk” transmitters will encounter similarly “at-risk” recipients. Clinical trial design should take into account the genetically defined individual differences in both susceptibility and transmissibility to better understand puzzling outcomes. Knowledge of population-specific host factors might also help identify what protective effects are attributable to the vaccine or to the host genotype.

Perhaps the STEP trial outcome signals that a step back is needed to seek more illumination on correlates of protection and susceptibility, rather than initiating trials of broadly similar vaccines (24). Additional studies on HIV-1 vaccine cohorts, coordinated with studies of natural infection, might yield useful information about genetic factors influencing both variable vaccine responses and variable susceptibility to infection. Do titers of antibodies to common viral and nonviral

pathogens, including Ad5, correlate with anti-gp120 antibody titers and HIV-1 infection status? What host genetic factors correlate with strong and weak antibody responses? HIV-1 vaccine research must finally step away from its roots in empiricism and embrace new discoveries in immunology and host genetics (24–26).

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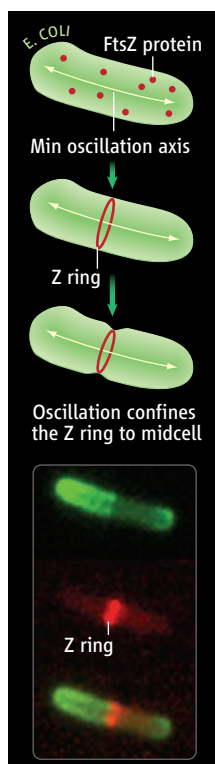
## BIOCHEMISTRY

# Tinkering with Acellular Division

Joe Lutkenhaus

One way to understand a complex biological process is to reconstitute it from purified components. Among many notable successes are the initiation of bacterial DNA replication, protein secretion in prokaryotic and eukaryotic cells, and assembly of the mitotic spindle that segregates chromosomes during eukaryotic cell division. For many other complex processes, reconstitution seems feasible, but for cytokinesis—the last stage of cell division in which the cytoplasm is divided to produce two daughter cells—this seems a formidable task. One might assume that a cell would be necessary to reconstitute such a phenomenon. However, two papers in this issue—by Osawa *et al.* on page 792 (1) and by Loose *et al.* on page 789 (2)—indicate that bacterial cytokinesis may be achievable in a cell-free system.

Cytokinesis in almost all bacteria and archaea, and in some organelles (chloroplasts and some mitochondria), uses a cytoskeletal element called the Z ring (3), which consists of filaments of polymerized FtsZ protein (see the figure), the bacterial homolog of the eukaryotic cytoskeletal protein tubulin (4). The Z ring provides a scaffold to recruit at least 10 additional proteins required for cytokinesis, many of which link the Z ring to synthesis of a new cell wall that accompanies formation of the septum. Though



**Simple and elegant.** The oscillation of Min proteins from end to end in a bacterium confines the Z ring (composed of FtsZ protein filaments) to the midcell region, where the final stage of cell division will occur. (Inset) Fluorescently labeled MinD (green) and FtsZ (red) are shown in an *E. coli* cell. [Reprinted from (18) with permission from Wiley-Blackwell]

conserved in most bacteria, these proteins are not present in the simplest bacteria that lack a cell wall. In these bacteria, only FtsZ is found, raising the question of whether it is sufficient for constriction of a bacterium lacking a rigid cell wall.

FtsZ does not attach directly to the bacterial membrane but uses a short, conserved carboxyl-terminal tail to bind to other membrane-bound proteins (5). Even in bacteria that lack a cell wall, this FtsZ tail is conserved, suggesting that some

as yet unknown protein is involved. To circumvent this requirement for membrane attachment in a reconstituted system, Osawa *et al.* replaced the conserved tail with a fluorescent protein and an amphipathic helix borrowed from the bacterial peripheral membrane protein MinD. After mixing this fusion protein with phospholipid vesicles and guanosine 5'-triphosphate (GTP, needed for polymerization of FtsZ), they observed Z rings moving within cylindrical tubes of lipid. Coalescence of faint rings into brighter rings led to partial constriction of the tube. With limiting GTP, constrictions at the Z rings dis-

Self organization of proteins involved in bacterial cell division is demonstrated in vitro.

appeared as the constrictions were resorbed. The study thus reveals that a dynamic Z ring can produce a force capable of initiating a constriction and argues that FtsZ itself (provided it is attached to the membrane) can assemble the Z ring.

FtsZ filaments are thought to be too short to form a complete ring (6). One possibility is that the ring is built through lateral interaction of the filaments. Also, the coalescence of faint rings to form the brighter rings that cause constriction suggests that lateral interactions between filaments may be involved. This is somewhat controversial as no contiguous ring of filaments is observed by electron tomography, a technique with high resolution (7). Perhaps not all filaments in the ring are preserved with this technique or membrane-tethered filaments are brought together by membrane distortions caused by their lipid anchors.

In the dividing bacterial cell, a Z ring is present long before constriction begins (8, 9). This suggests that one of the roles for the additional cell division proteins is regulatory—to hold the Z ring in check until the appropriate signal for constriction is received. Spatial regulation ensures that the Z ring forms at the cell center (10, 11). In the model bacterium *Escherichia coli*, an important component of spatial regulation is a rapid oscillator that shuttles an inhibitor of Z ring assembly between the ends of the cell. This oscillation occurs many times during the time course of a cell division cycle, resulting in a time-averaged concentration of the inhibitor that is lowest at midcell. The oscillator consists of an adenosine triphosphatase (ATPase) (MinD) and an activator (MinE). The dynamic behavior of these proteins results from the ATP-dependent accumulation of MinD on the membrane followed by its recruitment of MinE (12, 13).

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