(Fig. 3) are small. This change in temperature does not affect the first arrival, which means that the travel time of the first arrival cannot be used to infer any possible small change in velocity due to a 5°C temperature difference. The late time window (Fig. 3, bottom inset) shows a clear time shift of the waveforms.

For each change of ±5°C in temperature, the change in the velocity is inferred from Eq. 10 using 20 different time windows of the coda waves, each with a duration of 0.1 ms. The mean and variance of the velocity change (Fig. 4) is inferred from the estimates of the velocity change in the different time windows. The relative velocity change is of the order of 0.1% for a temperature change of ±5°C with an error of about 0.02%.

During the heating phase, the velocity change is constant for temperatures below 75°C. Above that temperature, the velocity change increases during heating (Fig. 4). The acoustic emissions correlate with the increased value of the velocity change at 75°C (14). During the cooling phase, the velocity change is constant and there are no acoustic emissions. When the sample is heated again to a temperature of 90°C, the velocity change does not increase dramatically around 75°C and there are no acoustic emissions (15). In order to test whether the transducer coupling and the presence of the heating coil played a role, we repeated the experiment with an aluminum sample. In that case the velocity change is constant both during heating and cooling.

The acoustic emissions and the change in the velocity gradient occur only in a pristine sample during heating [the Kaiser effect (14)] and are due to the irreversible formation of fractures by differential thermal expansion (16) of the minerals in the sample. This indicates that the velocity change is due to two different mechanisms. The first is a reversible change in velocity due to the change in bulk elastic constants with temperature. The second mechanism is associated with irreversible changes in the sample that generate acou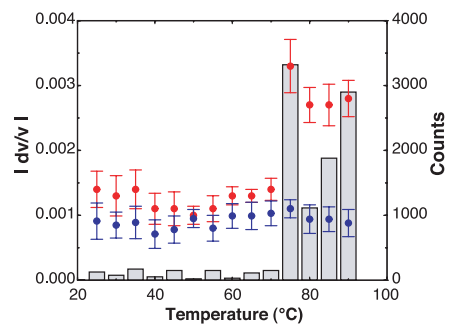stic emissions. The damage done to the sample leads to a greater change in the seismic velocity with increasing temperature.

These measurements could be carried out because of the extreme sensitivity of coda wave interferometry to changes in the medium. This technique makes it possible to infer the nonlinear dependence of the velocity on temperature that is associated with irreversible damage to the granite sample.

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Fig. 4. The absolute value of the relative velocity change for a 5°C increase and 5°C decrease, red and blue symbols, respectively, as a function of the highest temperature during the change. The histograms shows the count of acoustic emissions.

Adaptive Immune Response of Vγ2Vδ2+ T Cells During Mycobacterial Infections

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To examine the role of T cell receptor (TCR) in γδ T cells in adaptive immunity, a macaque model was used to follow Vγ2Vδ2+ T cell responses to mycobacterial infections. These phosphoantigen-specific γδ T cells displayed major expansion during *Mycobacterium bovis* Bacille Calmette-Guérin (BCG) infection and a clear memory-type response after BCG reinfection. Primary and recall expansions of Vγ2Vδ2+ T cells were also seen during *Mycobacterium tuberculosis* infection of naïve and BCG-vaccinated macaques, respectively. This capacity to rapidly expand coincided with a clearance of BCG bacteremia and immunity to fatal tuberculosis in BCG-vaccinated macaques. Thus, Vγ2Vδ2+ T cells may contribute to adaptive immunity to mycobacterial infections.

The majority of circulating γδ T cells in humans express a TCR heterodimer comprised of Vγ2 and Vδ2 segments. Recent in vitro studies have demonstrated that human Vγ2Vδ2+ T cells recognize small organic phosphate antigens (1–6) from microbes and other nonpeptide molecules such as alkylamines and aminobisphosphonates (7, 8). The recognition of these nonpeptide antigens by Vγ2Vδ2+ T cells does not require antigen processing or presentation by major histocompatibility complex (MHC) or CD1 molecules (9). *Mycobacterium tuberculosis*–induced tuberculosis remains a leading cause of morbidity and mortality among infectious diseases. Immune correlates of protection against tuberculosis remain poorly characterized in humans. De-

tailed analyses of $V\gamma2V\delta2^+$ T cell responses and their potential role in microbial infections have not been well documented in humans [see review, (10)], despite compelling evidence that murine $\gamma\delta$ T cells provide protection against a range of infections (11–16). The capability of $V\gamma2V\delta2^+$ T cells to mount a memory response after microbial reinfection or reactivation has not been demonstrated. Relevant studies in mice cannot be performed because murine $\gamma\delta$ T cells do not express the homolog of the $V\gamma2V\delta2^+$ TCR, and there is no functional equivalent for these cells, so far, identified in mice. We reasoned that nonhuman primates could provide a good model in which to determine the nature of the immune responses of $V\gamma2V\delta2^+$ T cells in a mycobacterial infection.

To examine a primary $\gamma\delta$ T cell response during mycobacterial infection, macaques were inoculated with BCG (17) and assessed for changes in their $\gamma\delta$ T cell repertoire (18). Striking expansions of circulating $V\gamma2V\delta2^+$ T cells were detected in the blood of these monkeys after BCG inoculation, whereas there was no apparent increase in other circulating $V\gamma^+$ or $V\delta^+$ T cells (Fig. 1A). The percent of peripheral blood $CD3^+$ T cells that were $V\gamma2V\delta2^+$ and the absolute number of circulating $V\gamma2V\delta2^+$ T cells increased by up to 25-fold and as much as a 200-fold, respectively (Fig. 1A). The expansion of $V\gamma2V\delta2^+$ T cells was most evident 3 to 5 weeks after BCG inoculation (Fig. 1A). The expansion of circulating $V\gamma2V\delta2^+$ T cells in the infected macaques indicated the development of primary response of these cells during a mycobacterial infection.

$V\gamma2V\delta2^+$ T cells compose the majority of the human $\gamma\delta$ T cell population in the circulation. We next examined whether macaque $V\gamma2V\delta2^+$ T cells in tissue compartments had expanded during a BCG infection. An increase in pulmonary $V\gamma2V\delta2^+$ T cells was evident after intravenous BCG inoculation (Fig. 1B). Expansion of the $V\gamma2V\delta2$ T cell subpopulation was also apparent within intestinal mucosae (Fig. 1B). Despite this expansion in blood and tissue sites, only a subtle expansion of $V\gamma2V\delta2^+$ T cells was observed in organized lymph nodes of the same monkeys. These findings suggest that the $V\gamma2V\delta2^+$ T cells in mucosal but not

peripheral lymph node tissues could expand in response to a mycobacterial infection. The expansion of $V\gamma2V\delta2^+$ T cells in these mucosal areas may result from their local proliferation in response to the BCG infection. In addition, $V\gamma2V\delta2^+$ T cells may also accumulate in these tissue sites through the recruitment from the circulating pool of $\gamma\delta$ T cells.

The hallmarks of memory T cell responses include their antigen-specific persistence after the initial infection and the rapid and prolonged recall responses upon reinfection. To determine whether $V\gamma2V\delta2^+$ T cell responses possessed the capacity for immunological memory, macaques that had previously been infected with BCG were inoculated again with BCG and were assessed for memory $V\gamma2V\delta2^+$ T cell responses. As early as 4 to 6 days after the second BCG inoculation, a marked expansion

of $V\gamma2V\delta2^+$ T cells was apparent in the blood of the monkeys (Fig. 2). The $V\gamma2V\delta2^+$ T cell expansions during the BCG reinfection were 2 to 9 times as large as those seen during primary infection (Fig. 2). The expansion of these cells persisted for as long as 7 months after the second BCG inoculation (Fig. 2). Analyses of TCR sequences revealed that some of the $V\gamma2V\delta2^+$ T cell clones that expanded during primary BCG infection were also identified in the $V\gamma2V\delta2^+$ T cell subpopulation that expanded after reinfection [see supplemental material (19)]. These molecular analyses provide evidence that $V\gamma2V\delta2^+$ T cells that underwent polyclonal expansion during a primary BCG infection can mount a memory or recall response after a secondary BCG infection.

Given that $V\gamma2V\delta2^+$ T cells contribute to adaptive immune responses, it seems likely that the clonally expanded cells that emerge after
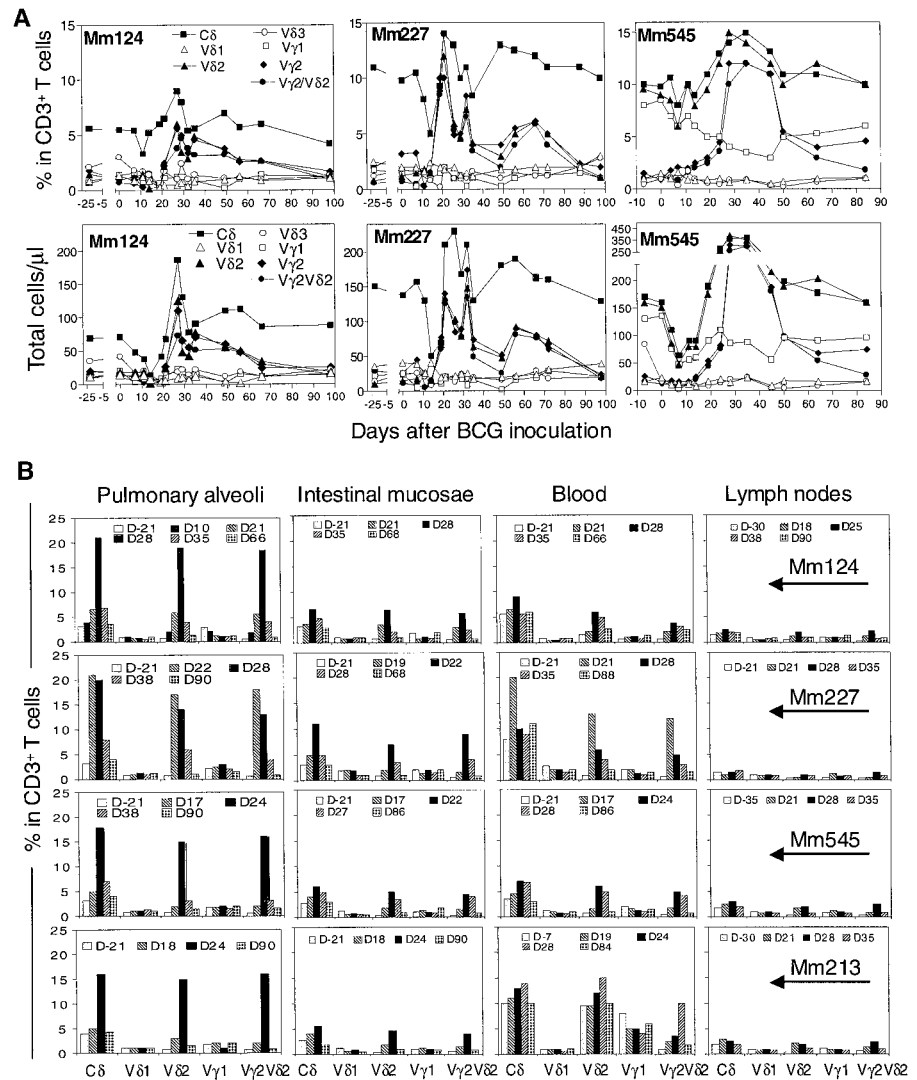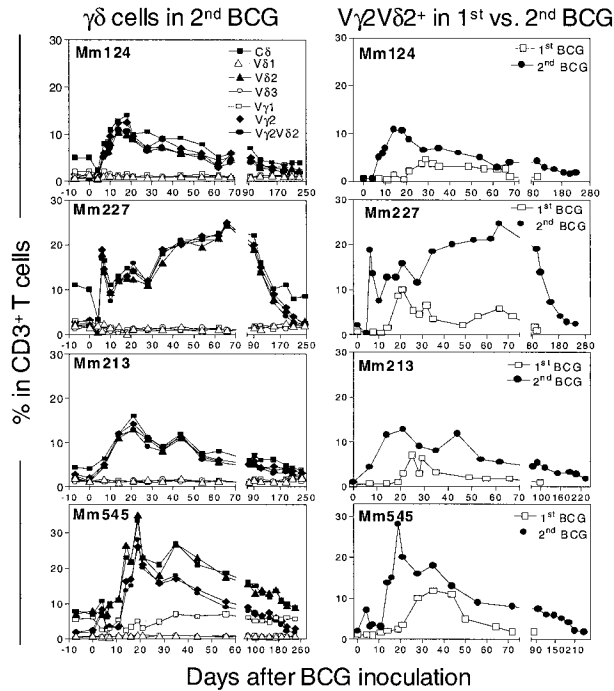

Fig. 1. BCG infection induced a major expansion of $V\gamma2V\delta2^+$ T cells in the macaques. (**A**) Changes in relative and absolute numbers of $CD3^+$ peripheral blood lymphocytes (PBL) that are $V\gamma^+$, $V\delta^+$, or $V\gamma2V\delta2^+$ T cells after BCG inoculation. (**B**) A major expansion of $V\gamma2V\delta2^+$ T cells in pulmonary alveoli and intestinal mucosae but not in lymph nodes of the macaques. Mm, rhesus monkeys (*Macaca mulatta*). The monkeys, 3 to 15 years of age, were inoculated intravenously with 10^6 CFU of BCG.

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Fig. 2. BCG reinfection induced a recall expansion of circulating Vγ2Vδ2+ T cells. The left panel shows a rapid increase in γδ+ T cells in CD3+ PBL after a second BCG inoculation, whereas the right panel shows that the responses of circulating Vγ2Vδ2+ T cells after BCG reinfection were greater in magnitude and longer in duration than the response of these cells after the first BCG infection. BCG reinfection of the monkeys that recovered from the primary active infection was done by intravenous inoculation of them with 10^8 CFU of BCG 3 to 5 months after the first BCG inoculation.

kinetics of BCG CFU counts and Vγ2Vδ2+ T cells in BCG-infected macaques suggest that Vγ2Vδ2+ T cells contribute to the immune containment of avirulent mycobacterial infections in macaques, and these findings are consistent with the antimicrobial activities of γδ T cells observed in mice (11–16).

Lastly, we sought to determine whether the adaptive immune responses of Vγ2Vδ2+ T cells seen in the BCG infection could also be identified in *M. tuberculosis* infection (19). After a *M. tuberculosis* aerosol challenge (exposure to aerosolized *M. tuberculosis*), the naïve macaques exhibited a primary expansion of Vγ2Vδ2+ T cells in the bronchoalveolar lavage (BAL) fluid (Fig. 4A). A rapid recall expansion of Vγ2Vδ2+ T cells was also apparent in BAL fluid after a *M. tuberculosis* aerosol challenge of the BCG-vaccinated macaques (Fig. 4A). Similar to what was observed after the pulmonary BCG exposure (unpublished observations), *M. tuberculosis* aerosol challenge did not induce an apparent expansion of Vγ2Vδ2+ T cells in the blood of naïve and BCG-vaccinated macaques in the acute infection. The rapid recall expansion of alveolar Vγ2Vδ2+ T cells in the BCG-vaccinated, *M. tuberculosis*-infected macaques coincided with the development of protective immunity to the acutely fatal form of tuberculosis (Fig. 4B). The BCG-vaccinated macaques remained clinically healthy during a 2.5-month period of follow up after *M. tuber-*

BCG infection were involved in the control of active BCG replication in the macaques. To address this possibility, we monitored active BCG infection by quantitating BCG colony counts in the blood (20) and determined the kinetics of Vγ2Vδ2+ T cell expansions during the course of the BCG infection. The expansion of Vγ2Vδ2+ T cells was associated with a clearance of detectable BCG bacteremia (Fig. 3). Our recent studies suggest that the decline of
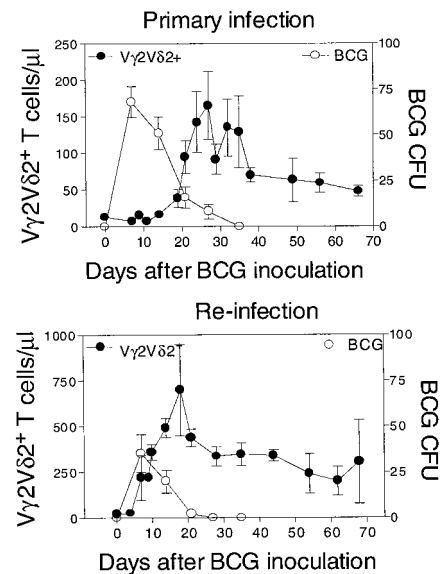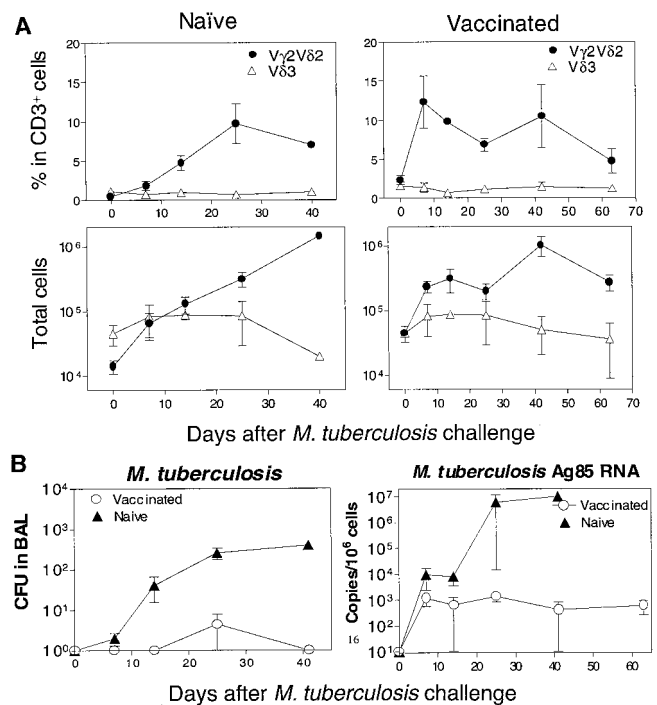
BCG colony-forming units (CFU) in the blood is dependent on the immune competence, because the enhanced T cell suppression during simian immunodeficiency virus–BCG (SIV-BCG) coinfection results in a prolonged BCG bacteremia in the blood (20, 21). Thus, the

Fig. 3. Vγ2Vδ2+ T cell expansion was associated temporally with the decreasing trend of active BCG infection. Shown is the kinetics of peripheral blood BCG CFUs (ml) and Vγ2Vδ2+ T cell expansions after the first (**top**) and second (**bottom**) BCG infection. Data shown are the means of values with the error bars of SEM from four infected animals.

Fig. 4. Rapid recall expansion of Vγ2Vδ2+ T cells after *M. tuberculosis* aerosol challenge was associated with immunity to acutely fatal tuberculosis in BCG-vaccinated monkeys. (**A**) *M. tuberculosis* aerosol challenge induced primary and recall expansions of alveolar Vγ2Vδ2+ T cells in four naïve (left) and four BCG-vaccinated (right) macaques, respectively. Both percentage and absolute numbers of alveolar Vγ2Vδ2+ T cells are shown after the *M. tuberculosis* aerosol challenge. (**B**) A reduced number of viable BCG (CFU/ ml) and Ag85 mRNA in BAL cells of BCG-vaccinated monkeys. The naïve macaques died from acutely fatal tuberculosis on days 25, 28, 38, and 41 after the *M. tuberculosis* aerosol challenge. Necropsy showed miliary tu-

berculosis in the lung, liver, spleen, and kidney of the dead macaques. The BCG-vaccinated macaques remained clinically healthy during a 2.5-month follow up after *M. tuberculosis* aerosol challenge (P < 0.001). The eight monkeys, all of which were 2 years of age, were challenged with 400 to 500 *M. tuberculosis* organisms by aerosol route (21). The BCG vaccination was done by intravenous inoculation of 10^6 CFU 6 weeks before the *M. tuberculosis* aerosol challenge. Quantitation of *M. tuberculosis* Ag85B mRNA was done with the use of real-time quantitative polymerase chain reaction (PCR) (21).

culosis challenge. In contrast, the unvaccinated macaques developed lethargy, anorexia, and wasting; they subsequently died and they showed evidence of miliary tuberculosis 4 to 6 weeks after *M. tuberculosis* aerosol challenge. We realize that the BCG-vaccinated, *M. tuberculosis*-infected monkeys may progress to a subclinical form of tuberculosis, because they did exhibit detectable *M. tuberculosis* and its mRNA in BAL cells (Fig. 4B). Nevertheless, this potential outcome would not negate our observation that a rapid recall response of Vγ2Vδ2$^+$ T cells coincided with immune protection against the acutely fatal tuberculosis in monkeys.

Our studies provide strong evidence that Vγ2Vδ2$^+$ T cells, like αβ$^+$ T cells, contribute to adaptive immune responses in mycobacterial infections. The adaptive immune responses of Vγ2Vδ2$^+$ T cells are indeed driven by BCG nonpeptide antigens (*19*). The contribution of these cells to vaccine protection against tuberculosis was demonstrated in the juvenile rhesus model. BCG-mediated protection against the fatal form of tuberculosis has been reported in children, although its protective efficacy for chronic pulmonary tuberculosis in adults and monkeys is controversial (*22–29*). It seems that the antigen specificity, TCR diversity, and recall features of Vγ2Vδ2$^+$ T cells separate this γδ T cell subset from innate cells including peripheral mononuclear cells (PMN), monocytes, and natural killer (NK) cells as well as those γδ T cells that express invariant γδ TCR (*30*, *31*). The unique ability of Vγ2Vδ2$^+$ T cells to mount rapid and large expansions in mycobacterial infections suggests that vaccine-elicited Vγ2Vδ2$^+$ T cell immunity may be both possible and useful. Thus, Vγ2Vδ2$^+$ T cells may broadly contribute to both innate and acquired immunity against microbial infections.

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An mRNA Surveillance Mechanism That Eliminates Transcripts Lacking Termination Codons

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Translation is an important mechanism to monitor the quality of messenger RNAs (mRNAs), as exemplified by the translation-dependent recognition and degradation of transcripts harboring premature termination codons (PTCs) by the nonsense-mediated mRNA decay (NMD) pathway. We demonstrate in yeast that mRNAs lacking all termination codons are as labile as nonsense transcripts. Decay of "nonstop" transcripts in yeast requires translation but is mechanistically distinguished from NMD and the major mRNA turnover pathway that requires deadenylation, decapping, and 5′-to-3′ exonucleolytic decay. These data suggest that nonstop decay is initiated when the ribosome reaches the 3′ terminus of the message. We demonstrate multiple physiologic sources of nonstop transcripts and conservation of their accelerated decay in mammalian cells. This process regulates the stability and expression of mRNAs that fail to signal translational termination.

Eukaryotes have evolved surveillance mechanisms that are intimately linked to translation to eliminate errors in mRNA biogenesis. The decay of transcripts containing PTCs by the NMD pathway effectively prevents expression of deleterious truncated proteins. In prokaryotes, protein products encoded by transcripts lacking termination codons are marked for degradation by the addition of a COOH-terminal tag encoded by tmRNA (*1, 2*). Thus, both the presence and context of translational termination can regulate gene expression. In order to determine whether the presence of translational termination influences mRNA stability, we assayed PGK1 tran-

scripts in *Saccharomyces cerevisiae* derived from the following constructs (*3*): wild-type PGK1 (WT-PGK1), a nonsense form of PGK1 harboring a PTC at codon 22 [PTC(22)-PGK1], and nonstop-PGK1 that was created by removing the bona fide termination codon and all in-frame termination codons in the 3′ UTR (untranslated region) from the WT-PGK1 transcript. Nonstop-PGK1 transcripts were as labile as their nonsense-containing counterparts (Fig. 1). At least three *trans*-acting factors (Upf1p, Upf2p, and Upf3p) are essential for NMD in *S. cerevisiae* (*4, 5*). Remarkably, nonstop transcripts were not stabilized in strains lacking Upf1p, distinguishing the pathway of decay from NMD (Fig. 1).

The turnover of normal mRNAs requires deadenylation followed by Dcp1p-mediated decapping and degradation by the major 5′-to-3′ exonuclease Xrn1p (*6, 7*). NMD is distinguished in that these events occur without prior deadenylation (*8, 9*). Nonstop-PGK1 transcripts showed rapid decay

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