larity to the profiles described here in lesions (*9*), we believe the lymphokine mRNAs in lesions reflect the pattern of T cell subsets associated with resistant and susceptible forms of the disease.

Studies of immune responses to leishmaniasis in BALB/c mice have demonstrated that $CD4^+$ T cells capable of adoptively transferring resistance to infection produce predominantly IL-2 and IFN-γ in vitro, whereas those that induce exacerbation and more rapid lesion formation produce primarily IL-4 and IL-5 (*10*). Treatment of susceptible mice with antibodies to IL-4 resulted in increased IFN-γ production with a concomitant increase in the host resistance to the *Leishmania major* parasites (*11*). Although we cannot differentiate whether the cytokine patterns in the leprosy lesions are the cause or result of disease, the extensive studies in murine models suggest that the cytokine patterns are critical to the development of the characteristic lesions in leprosy. Such cytokine patterns are likely to contribute to the pathogenesis of other chronic infectious diseases of man. Further long-term horizontal studies of affected individuals with and without treatment by immunotherapy should define the dynamics of cytokine patterns in the pathogenesis of human infections.

Developing new and improved vaccines (*12*) requires greater understanding of the complex immunoregulatory mechanisms that determine protective immunity, unresponsiveness, and tissue damage. The patterns of lymphokines and cytokines produced at the sites of infection, together with specific cell-mediated cytotoxic activities, are most likely to determine the outcome of the host-pathogen interaction. Because several of the cytokines found particularly in self-healing lesions can produce tissue damage, as well as enhanced microbicidal activity (such as TNF-α, IFN-γ, and LT), it is appropriate to wonder about the extent to which the pathogenesis of these infectious diseases, such as the destruction of the nerves in leprosy or of the lungs in tuberculosis, is the price to be paid by the host for protection.


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14. We thank J. Zack for assistance with the purification of RNA from lesions. Supported by grants from the NIH (AI 22553, AR 40312, AI 07118, AI 20111, and AI 26491), the United Nations Development Programme–World Bank–World Health Organisation Special Programme for Research and Training in Tropical Diseases [Immunology of Leprosy (IMMLEP) and Therapy of Leprosy (THELEP)], the Heiser Trust, the Sasakawa Memorial Health Foundation, and the Dermatologic Research Foundation of California.

7 May 1991; accepted 5 August 1991


# Differing Lymphokine Profiles of Functional Subsets of Human CD4 and CD8 T Cell Clones


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**Functional subsets of human T cells were delineated by analyzing patterns of lymphokines produced by clones from individuals with leprosy and by T cell clones of known function. CD4 clones from individuals with strong cell-mediated immunity produced predominantly interferon-γ, whereas those clones that enhanced antibody formation produced interleukin-4. CD8 cytotoxic T cells secreted interferon-γ. Interleukin-4 was produced by CD8 T suppressor clones from immunologically unresponsive individuals with leprosy and was found to be necessary for suppression in vitro. Both the classic reciprocal relation between antibody formation and cell-mediated immunity and resistance or susceptibility to certain infections may be explained by T cell subsets differing in patterns of lymphokine production.**


T CELLS HAVE BEEN DIVIDED INTO subsets based on expression of different surface antigens. $CD4^+$ T cells function as helper cells in antibody formation and mediate delayed-type hypersensitivity (DTH), a form of cell-mediated immunity. $CD8^+$ T cells encompass cytotoxic T lymphocytes (CTLs) and antigen-specific T suppressor ($T_S$) cells. Murine $CD4^+$ T cells effecting different immunological functions have been further subdivided according to distinct, nonoverlapping patterns of lymphokine production (*1*). $CD4^+$ T cells involved in DTH responses (designated $T_H1$ cells) produce interleukin-2 (IL-2), interferon-γ (IFN-γ), lymphotoxin, and other lymphokines but little IL-4 or IL-5. Reciprocally, murine $CD4^+$ T helper cell clones that regulate antibody production ($T_H2$ cells) preferentially produce IL-4, IL-5, and IL-6 but little IL-2 or IFN-γ. However, most human $CD4^+$ clones derived from healthy donors produce IL-2, IFN-γ, IL-4, IL-5, granulocyte-macrophage colony-stimulating factor (GM-CSF), or combinations thereof that have been difficult to relate to the murine $T_H1$ and $T_H2$ subsets or to specific biological function (*2*). Human T


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cell clones (*3*), many from allergic individuals, have been described with the lymphokine profile of murine $T_H2$ cells. A difference between murine and human studies is that the mice studied were hyperimmunized, which suggested to us that it might be informative to analyze specific T cell clones from individuals responding to an infectious pathogen.

Leprosy presents a model for exploring functional T cell subsets in humans because it is a chronic infectious disease that presents, not as a single clinical entity, but as a clinical and immunological spectrum. The resistant tuberculoid form has strong DTH, few lesions, and few bacilli, whereas the susceptible lepromatous form has multiple skin lesions, large numbers of bacilli, high antibody levels, and selective unresponsiveness of cell-mediated immunity to antigens of *Mycobacterium leprae* (*4*). $CD4^+$ cells predominate in tuberculoid lesions, and $CD8^+$ T cells are the major subset in lepromatous lesions (*5*). Of $CD4^+$ cells cultured directly from tuberculoid lesions, one in 60 respond to *M. leprae* antigens (*6*). $CD8^+$ cells from lepromatous lesions or blood do not proliferate in response to antigen but can be activated by antigens found uniquely on *M. leprae* to suppress proliferative responses of $CD4^+$ cells (7).

We have examined the profiles of lymphokines produced by T cell clones derived from individuals across the clinical spectrum of leprosy and their familial contacts and compared them to lymphokine profiles of cells specific for other antigens bearing the same surface phenotype but differing in function (Table 1). The $CD4^+$ clones from tuberculoid individuals proliferated in response to *M. leprae* antigens, whereas the $CD8^+$ clones from unresponsive lepromatous individuals did not proliferate and suppressed the proliferative response of $CD4^+$

**Table 1.** Lymphokine production by $CD4^+$ and $CD8^+$ clones stimulated by immobilized antibodies to CD3. The $CD8^+$ cytotoxic clones specific for HLA-B27 have been reported (*8*). Percent cytotoxicity was determined by $^{51}Cr$ release of labeled target cells. HA1.75 is a Flu peptide (amino acids 307 to 319)–reactive clone (*31*). *Mycobacterium leprae*– and tetanus toxoid–reactive clones were established by limiting-dilution cloning (*32*). The $CD4^+$ clones were stimulated by the specific antigen in the presence of HLA-DR–matched antigen-presenting cells, and incorporation of an 8-hour pulse of $^3H$-labeled thymidine was measured at 72 hours. The $CD4^+$ and $CD8^+$ $T_S$ clones were generated from lesions and peripheral blood (PBL) as described (7). Suppressive activity of the $T_S$ clones was measured by the inhibition of [$^3H$]thymidine incorporation by *M. leprae* antigen–stimulated CD4 cells (7). CD4 and CD8 receptor amounts on the clones were measured by flow cytometry. For lymphokine production, T clones ($1 \times 10^5$) in 1 ml of Iscove's medium and 10% human AB serum were added to 24-well plates coated with 2.5 μg of antibody to CD3 (OKT3). We purified T cell clones over Ficoll-Paque (Pharmacia) before use to remove residual antigen and feeder cells. Supernatants from the stimulated cells were harvested 18 to 20 hours later and stored at −20°C. T cell–depleted antigen-presenting cells were included for assay of IL-2 production. Lymphokine amounts in the various supernatants (in picograms per milliliter) were assayed by enzyme immunoassay as follows: IFN-γ and IL-6, Amgen Biologicals; IL-4 and GM-CSF, Genzyme; and IL-2 and TNF-α, T-Cell Sciences (Cambridge, Massachusetts). IL-5 (*33*) was measured by monoclonal antibody–based immunoenzymetric assay. T-leprosy, tuberculoid leprosy; and L-leprosy, lepromatous leprosy.

| T cell subset | Clone | Derived from | Specificity | Function (reference) | IFN-γ | IL-4 | IL-5 | IL-6 | IL-2 | GM-CSF | TNF-α |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Type 1 CD4 | 59.5. | PBL contact | *M. leprae* | DTH? (*32*) | 14,832 | 42 | 0 | 0 | 180 | 2,618 | 1,540 |
| | 59.3. | | | | 8,000 | 0 | 0 | 0 | 200 | | 30 |
| | 36.1. | | | | 8,500 | 250 | | 0 | 234 | | |
| | 63.5. | | | | 2,500 | 0 | 0 | 40 | 156 | | |
| | 61.4. | | | | 6,200 | 0 | 0 | 0 | 125 | 40 | 540 |
| | 3.4. | | | | 4,800 | 0 | | 0 | 210 | 80 | 1,000 |
| | 3.5. | | | | 8,500 | 0 | | 0 | 165 | 800 | 1,000 |
| | L-9 | Lesion T-leprosy | | | 5,800 | 0 | | 0 | | | |
| | L-11 | | | | 4,230 | 0 | 0 | 0 | | 432 | 50 |
| | L-12 | | | | 7,217 | 0 | 0 | 470 | | 1,870 | 300 |
| | L-22 | | | | 2,400 | 0 | | 0 | | | |
| | L-23 | | | | 6,634 | 0 | 0 | 170 | | 36 | 140 |
| | HA1.75 | PBL | Flu | | 10,800 | 450 | 22 | 0 | 125 | 400 | 1,000 |
| | RB-TT9 | | Tetanus | | 1,500 | 0 | | 0 | 220 | 38 | |
| Type 2 CD4 | RB-TT1 | PBL | Tetanus | B cell help | 1,500 | 1,500 | | 0 | | 160 | 180 |
| | RB-TT3 | | | | 900 | 1,100 | 410 | 0 | 100 | 22 | 0 |
| | RB-TT4 | | | | 0 | 7,000 | 1,100 | 0 | 100 | 120 | 300 |
| | RB-TT7 | | | | 345 | 8,142 | 7,532 | 0 | 100 | 1,000 | 245 |
| | RB-TT10 | | | | 1,449 | 7,060 | 7,532 | 0 | 112 | 6,334 | 250 |
| | RB-TT14 | | | | 0 | 1,000 | | 0 | | 40 | 1,000 |
| | RB-TT21 | | | | 0 | 700 | | 0 | 135 | 1,200 | |
| Type 2 CD4 $T_S$ | LL-8/9 | PBL, L-leprosy | *M. leprae* | Suppression | 98 | 7,899 | 717 | 0 | 1,250 | 5,388 | 4,000 |
| Type 1 CD8 | WG3 | PBL | HLA-B27 | Cytotoxicity (*8*) | 3,083 | 34 | 132 | 372 | | 40 | 490 |
| | WG8 | | | | 11,772 | 0 | 0 | 1,660 | 150 | 400 | 2,000 |
| | 5B | | | | 4,500 | 0 | 0 | 50 | 300 | 0 | |
| | 12B | | | | 1,500 | 0 | 0 | 60 | | 0 | 600 |
| | 4B | | | | 0 | 0 | 0 | 0 | | 16 | 0 |
| | 11B | | | | 2,500 | 0 | 0 | 65 | 125 | 0 | 400 |
| Type 2 CD8 | LL-16/10 | PBL, L-leprosy | *M. leprae* | Suppression (7) | 800 | 1,970 | 70 | 0 | 0 | 602 | 375 |
| | LL-7/2 | | | | 1,702 | 2,081 | 176 | 0 | 0 | 1,282 | 225 |
| | LL-14/7 | | | | 2,150 | 2,375 | 30 | 0 | 260 | 6,334 | 200 |
| | LL11 | | | | 2,000 | 4,500 | 1,831 | 0 | 140 | 20 | 0 |
| | LL19 | | | | 1,200 | 1,450 | 413 | 0 | | 200 | 240 |
| | JG5 | Lesion L-leprosy | | | 2,800 | 1,275 | 32 | 0 | 200 | 4,737 | 150 |
| | R-303 | | | | 1,250 | 1,050 | 32 | 20 | 300 | 1,250 | 720 |
| | R-101 | | | | 0 | 375 | | | | | |
| | R-107 | | | | 0 | 200 | | | | | |

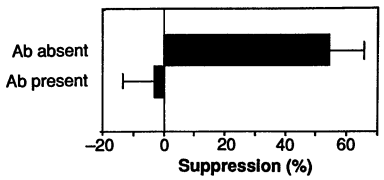


**Fig. 1.** Inhibition of antigen-induced suppression by antibody to IL-4. *Mycobacterium leprae*–specific $CD4^+$ clones ($1 \times 10^4$) were cultured with feeder cells ($1 \times 10^5$), *M. leprae* antigen (10 μg/ml), and $CD8^+$ $T_S$ clones ($1 \times 10^4$) in the presence or absence of antibody to IL-4 (1 μg/ml from the start of culture). Proliferation of the $CD4^+$ clones was measured by [$^3$H]thymidine incorporation on day 3. The data are presented as mean ± SE. Percent suppression was calculated as [100 − (counts per minute from antigen-containing cultures)/(counts per minute from antigen-containing cultures + $T_S$ cells) × 100].

clones to *M. leprae* antigen. We compared the lymphokines produced by 12 $CD4^+$ leprosy clones to 8 $CD4^+$ clones specific for tetanus toxoid and one for influenza hemagglutinin (Flu), as well as 9 lepromatous $CD8^+$ clones and 6 $CD8^+$ major histocompatibility complex (MHC) class I–restricted cytotoxic T lymphocytes specific for human leukocyte antigen (HLA)–B27 (*8*). To obviate the confounding production of cytokines by antigen-presenting cells, we stimulated the T cell clones with antibodies to CD3 (δ chain of the T cell receptor complex). All of the *M. leprae*–specific CD4 clones from antigen-responsive individuals or their contacts and the Flu clone produced IFN-γ, IL-2, and GM-CSF but little or no IL-4 or IL-5, similar to the pattern of cytokines characteristic of murine $T_H1$ cells. These cells lacked helper activity for antibody formation and were designated "Type 1" $CD4^+$ cells. The $CD4^+$ tetanus toxoid clones produced little IFN-γ and IL-2 but, with one exception, high levels of IL-4, IL-5, and GM-CSF, a pattern similar to murine $T_H2$ cells and were designated as "Type 2" $CD4^+$ cells. Two of the clones that produced substantial levels of IL-4 and IL-5 (RB-TT4 and 7) had helper activity for B cells (they induced immunoglobulin M production by the SKW6.4 human B cell line) (*9*). In contrast, four Type 1 $CD4^+$ clones producing IFN-γ (59.5, 61.4, 36.1, and HA1.75) (Table 1) failed to provide help for immunoglobulin production.

Despite the low amounts of IFN-γ and IL-2 (*10*) produced by most clones, these subsets showed essentially nonoverlapping patterns above minimal amounts of certain lymphokines. Antigen-specific human $CD4^+$ clones from strongly antigen-reactive donors can be classified into two functional subsets based on production of IFN-γ or IL-4 and IL-5, consistent with the findings for cytokine mRNAs present in leprosy lesions (*11*). Thus distinct human $CD4^+$ subsets, homologous to murine counterparts, exist in humans and can be distinguished by their cytokine profiles.

The nature and role of suppressor cells is controversial. With the possible exception of the CD28 surface marker associated with CTLs but not $T_S$ cells (*12*), it has been difficult to discriminate between $CD8^+$ CTLs and $T_S$ cells other than by their function and MHC restriction for antigen (CTLs are generally MHC class I–restricted, and the $T_S$ cells are MHC class II–restricted (*13*). The $CD8^+$ $T_S$ clones (seven out of nine) from lepromatous individuals produced predominantly IL-4 but little or no IL-6, IL-10, IL-5, or IFN-γ. In contrast, five out of six $CD8^+$ alloreactive CTL clones secreted IFN-γ, IL-6, and IL-10 but, with the exception of WG3, made no detectable IL-4 or IL-5. IL-10, a negative-regulatory lymphokine (*14*), was not produced by the $T_S$ clones (57 ± 55 pg, $n = 7$, compared to 982 ± 375 pg, $n = 6$, for the CTL clones; mean ± SEM), but its mRNA was found in lepromatous lesions (*11*), perhaps expressed by macrophages. Although IL-4 and IL-5 production are highly associated in mouse $T_H2$ cells, IL-5 was not produced in abundance by the IL-4–producing $CD8^+$ $T_S$ clones. Our data suggest that the human $CD8^+$ population can also be divided into two subsets: $CD8^+$ cytotoxic cells (Type 1) that produce no IL-4 but significant amounts of IFN-γ and IL-10 and $CD8^+$ $T_S$ cells (Type 2) that produce substantial amounts of IL-4.

IL-4 production by the $T_S$ cells was a necessary condition for suppression in this system because $CD8^+$ suppressor activity was inhibited in five of five clones by monoclonal antibodies to IL-4 (Fig. 1). We note that the single $CD4^+$ T cell clone (LL-8/9) derived from a lepromatous individual responded to *M. leprae*, not by secreting IFN-γ, but by producing a high level of IL-4 and suppressing the proliferative responses of other MHC class II–matched CD4 clones. CD4 $T_S$ cells have been reported in experimental leishmaniasis (*15*) and tumors (*16*), as well as in lepromatous leprosy (*17*). If IL-4 is a general mediator of this type of suppression, the apparent discrepancy in the literature between antigen-specific CD8 and CD4 suppressor cells can be readily reconciled. In mice, the relative balance of the $T_H1$ and $T_H2$ subsets of CD4 cells determines the outcome of the host response to several infectious pathogens: *Leishmania* (*18*), *Trichinella* (*19*), schistosome (*20*), and *Nippostrongylus* species (*21*). There is now evidence that IL-4 and IFN-γ function in reciprocal fashion and mutually inhibit their respective functions. IFN-γ is required for protection against intracellular pathogens. In *Leishmania major* infection of BALB/c mice, IL-4 derived from $T_H2$ clones leads to an exacerbated course, and conversely, treatment with antibodies to IL-4 before infection precipitates healing (*22*). In human filariasis, immunoglobulin E production to filarial antigens is mediated by IL-4 and down-regulated by IFN-γ (*23*).

A striking finding of the present results is the central role of IL-4 in discriminating between the functional subsets of both $CD4^+$ and $CD8^+$ T cell subpopulations and in suppression. In addition to its necessary role in differentiation and immunoglobulin class switching of B cells (*24*), IL-4 is the major growth factor for $T_H2$ cells (*25*), down-regulates IL-2 receptors, and blocks IL-2–dependent proliferation (*26*) and IFN-γ production by human peripheral blood mononuclear cells (*27*). IL-4 is also a potent inhibitor of macrophage functions, reducing their production of tumor necrosis factor–α (TNF-α), IL-1, prostaglandins, and reactive oxygen intermediates (*28*). Although IL-4 can mediate suppression, it remains to be established whether IL-4 alone is sufficient. It should be noted that other cytokines known to down-regulate Type 1 T cell functions, particularly IL-10 (*1*) and transforming growth factor–β (*29*), may be involved in various instances as well.

The present findings suggest an explanation for the classical immunological dichotomy between the development of humoral immune responses and those of cell-mediated immunity, phenomena described as "immune deviation" or "split tolerance" (*30*). The observations of reciprocal relations between antibodies or Type 2 T cell functions and Type 1 T cells in many infectious disease models can in part be explained by the action of IL-4, acting alone or in concert with other cytokines, not only to enhance antibody formation but also to depress multiple facets of cell-mediated immunity required for protection. The data encourage the view that the differing functional activities of both human $CD4^+$ and $CD8^+$ T cell subsets can be discriminated and possibly predicted by analysis of their lymphokine profiles. These lymphokine profiles may be determinative of the outcome of specific infectious challenges to host defenses.

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34. We thank J. Lamb for the influenza hemagglutinin peptide–specific CD4 clone (HA1.75), J. Melancon-Kaplan for help in generating the *M. lepraе*–specific CD4$^+$ clones, A. D. Donnenberg for antibody to CD3 (OKT3), F. Finkelman and G. Thyphronitis for antibodies to IL-4, and R. Rees and the Immunology of Leprosy (IMMLEP) Committee of the United Nations Development Programme–World Bank–World Health Organization Special Programme for Research and Training in Tropical Diseases for the *M. leprae* antigen. The DNAX Research Institute is supported by Schering-Plough. Supported by NIH grants AI-07118, 20111, 26491, and 22553.

7 May 1991; accepted 5 August 1991

# Cell Cycle Dependence of Laminar Determination in Developing Neocortex

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**The neocortex is patterned in layers of neurons that are generated in an orderly sequence during development. This correlation between cell birthday and laminar fate prompted an examination of how neuronal phenotypes are determined in the developing cortex. At various times after labeling with [$^3$H]thymidine, embryonic progenitor cells were transplanted into older host brains. The laminar fate of transplanted neurons correlates with the position of their progenitors in the cell cycle at the time of transplantation. Daughters of cells transplanted in S-phase migrate to layer 2/3, as do host neurons. Progenitors transplanted later in the cell cycle, however, produce daughters that are committed to their normal, deep-layer fates. Thus, environmental factors are important determinants of laminar fate, but embryonic progenitors undergo cyclical changes in their ability to respond to such cues.**

SEVERAL BRAIN REGIONS, INCLUDING the neocortex, segregate neurons with characteristic dendritic and axonal morphologies into distinct cellular layers. [$^3$H]thymidine "birthdating" studies of the developing cerebral cortex have shown that its six layers are generated in an orderly sequence. Neurons destined for the deepest layers leave the mitotic cycle first and migrate to form the cortical plate. Subsequently neurons of the more superficial layers are added, following an inside-out gradient of development (*1, 2*). This correlation between a neuron's birthday and its ultimate laminar fate suggests that the time at which a cell is generated may determine its identity in the cortex. Lineage studies using retroviral vectors indicate that early cortical progenitors can produce neurons in several layers (*3, 4*), presumably through asymmetric cell divisions (*4*). A sequential production of different neuronal phenotypes could result from progenitors that are intrinsically programmed first to produce deep-layer neurons and later upper-layer cells. Alternatively, multipotential progenitors or their daughters may respond to environmental cues that trigger the production of appropriate neuronal phenotypes. Transplantation studies can directly test the commitment of cells to their normal fates, thus distinguishing between cell-autonomous and environmental determinants of neuronal identity. Previous isochronic (*5, 6*) and heterochronic (*6*) transplants have shown that transplanted cortical neurons can migrate, differentiate, and form long-distance axonal connections, and have suggested that the layer to which a neuron migrates depends on the environment in which it goes through its final mitotic division (*6–8*). We examined the developmental potential of newly generated cortical neurons and their progenitors and the timing of laminar commitment by transplanting cells into host ferret brains at different times in the cell cycle.

[$^3$H]thymidine birthdating studies in the ferret (*2, 6*) show that neurons generated on embryonic day (E) 29 are normally destined for layer 6 and the subplate (*9*). To test the commitment of E29 cells to a deep-layer fate, we transplanted embryonic cells into postnatal host brains in which layer 2/3 neurons were being generated (*2, 6*). E29 cells were labeled in utero with [$^3$H]thymidine; cells developed undisturbed for 0 to 24 hours so that [$^3$H]thymidine-labeled cells could be caught in various stages of the cell cycle at the time of transplantation (Fig. 1A). Dissociated cells from the posterior (presumptive visual) cerebral wall were then injected into or near the occipital ventricular zone of a neonatal host ferret (*10*); hosts survived for at least 6 weeks, which allowed transplanted neurons to migrate and settle into a cortical layer. We first examined the developmental potential of newly generated postmitotic neurons, before migration. Cells were transplanted 24 hours after [$^3$H]thymidine labeling, an interval that allowed labeled progenitors to complete their current round of cell division before transplantation. Neurons committed to their normal fates should behave autonomously and migrate to the deep layers of the cortex, but if environmental cues determine laminar fate, transplanted neurons should adopt the host fate by migrating to layer 2/3 (Fig. 1B). As in previous heterochronic transplants (*6*), only a fraction of the cells labeled with [$^3$H]thymidine migrated into the host cortex (Fig. 1C); the remainder formed a small island of cells buried in the white matter at the injection site. However, those neurons that did migrate into the cortex were found in layer 6, appropriately for their birthday. In sum, over 85% of neurons transplanted after a 24-hour interval migrated to the infragranular layers (below layer 4), with the

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