tory response by the production of nuclear factor kappa B (NF-κB), interleukin-6 (IL-6), and tumor necrosis factor–α (TNF-α) (*29, 30*). Lastly, the catalytic antibody field has shown that antibodies are capable of much more complex chemistry than simple binding. It has not been previously thought that this potential for complex chemistry plays a role in their in vivo function. However, in light of our data, one must now consider that all antibodies have an innate catalytic potential that may be exploited for host protection.

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11. The issue of proximity makes a stoichiometric comparison between the effects of H_2O_2 in solution and H_2O_2 generated on the bacterial cell surface complicated in the case of antigen-specific antibodies. Therefore, antibody-generated H_2O_2 and H_2O_2 in solution were compared with antigen nonspecific antibodies.
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19. We presume that ozonolysis leads to ^{18}O isotope incorporation into the lactam carbonyl of **2** by trapping the presumed carbonyl oxide intermediate generated during ozonolysis by $H_2{}^{18}O$. We attribute the lack of isotope incorporation into **2** after oxidation by $^1O_2$* in phosphate buffer (PB) as being in accord with the known propensity of alkenes that contain no allylic hydrogens and at least one electron-donating atom α-to the olefin to form dioxetane intermediates which collapse to further products via a presumed retro [2+2] process (*20*).
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Maintenance of Serological Memory by Polyclonal Activation of Human Memory B Cells

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Production of antibodies can last for a lifetime, through mechanisms that remain poorly understood. Here, we show that human memory B lymphocytes proliferate and differentiate into plasma cells in response to polyclonal stimuli, such as bystander T cell help and CpG DNA. Furthermore, plasma cells secreting antibodies to recall antigens are produced in vivo at levels proportional to the frequency of specific memory B cells, even several years after antigenic stimulation. Although antigen boosting leads to a transient increase in specific antibody levels, ongoing polyclonal activation of memory B cells offers a means to maintain serological memory for a human lifetime.

Stimulation by antigen through the B cell receptor (BCR) followed by cognate T cell help drives proliferation and differentiation of antigen-specific naïve B lymphocytes into memory B cells and plasma cells (*1, 2*). Memory B cells carrying somatically mutated immunoglobulin (Ig) genes survive in secondary lymphoid organs in the absence of antigen (*3*) and mediate secondary immune responses upon rechallenge. In contrast, plasma cells are terminally differentiated, nondividing cells that home to spleen and bone marrow and are the main source of antibody, which they secrete at a high rate. Mouse plasma cells can be long-lived and are able to sustain antibody production for several months in the absence of memory B cells or antigen (*4, 5*). However, it is less likely that long-lived plasma cells produced during an immune response will maintain a constant supply of specific antibody over a human life-span, because even long-lived plasma cells would eventually need to be replenished over a human lifetime.

Whether persisting antigen is required to maintain serological memory remains debated (*6–8*). Antigen-driven proliferation and differentiation of memory B cell to short-lived plasma cells induces high levels of protective antibodies (*9*). Yet, if persistence of antigen was the only mechanism available to maintain antibody production, immunological memory would be limited to persisting antigens. We therefore searched for alternative mechanisms that might ensure sustained proliferation and differentiation of memory B cells, independently of persisting antigen.

Two types of polyclonal stimuli exist that can trigger B lymphocyte proliferation and differentiation in the absence of antigen: (i) those derived from microbial products, such as lipopolysaccharide or unmethylated single-stranded DNA motifs (CpG oligonucleotides), which stimulate B cells via TLR4 (Toll-like receptor 4) and TLR9, respectively (*10, 11*); and (ii) T cells activated by a third-party antigen, which stimulate B cells in a noncognate fashion via CD40 ligand and cy-

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tokine production, here referred to as bystander help (*2, 12*). We investigated whether such stimuli might preferentially trigger human memory B cells. Naïve B cells and two memory B cell subsets, carrying IgM (IgM memory B cells) or other isotypes due to class switching (switch memory B cells), were isolated from peripheral blood by sorting for expression of CD27 (*13*) and Ig isotypes (Fig. 1A), labeled with carboxyfluorescein diacetate succinimidyl ester (CFSE), and tested for their capacity to proliferate in response to CpG (Fig. 1B) (*14*). Under these conditions, naïve B cells did not divide, although they up-regulated CD69 and CD86 and increased in size (*15*). In contrast, IgM memory B cells underwent several rounds of division, whereas switch memory B cells proliferated in the presence of CpG and interleukin-15 (IL-15). $F(ab')_2$ fragments of antibody to human Ig (anti-Ig), used as surrogate antigen, failed to induce B cell proliferation, even in the presence of cytokines (*15*). However, anti-Ig strongly induced proliferation of naïve B cells in the presence of CpG and IL-15. We conclude that, in contrast to naïve B cells that depend on BCR signaling, memory B cells are activated by CpG and cytokines without need for BCR triggering.

Recently activated T cells migrate to B cell areas of secondary lymphoid tissues where they interact with B cells in a cognate, and possibly noncognate, fashion (*16*). To test the response of B cells to noncognate T cell help, we cultured CFSE-labeled B cell subsets in the presence of an alloreactive T helper clone, stimulated by appropriate allogeneic antigen-presenting cells. In these culture conditions, the T helper clone was unable to recognize B cells, thus providing bystander help in the absence of a cognate interaction (*12*). Although switch memory B cells divided extensively in response to this bystander T cell help, IgM memory B cells were less responsive, and naïve B cells remained unresponsive (Fig. 1C). Remarkably, the response of switch memory B cells was not enhanced by the addition of anti-Ig or CpG. We conclude that bystander T cell help is an optimal and sufficient trigger for initiating proliferation of human memory B cells.

After stimulation with CpG or T cell help, a fraction of proliferating memory B cells acquired phenotypic markers characteristic of plasma cells, namely, loss of CD20 (Fig. 2A) and up-regulation of CD138, CD126, and CD38 (*15*). These cells also secreted IgM, IgG, and IgA at a high rate (*14*). The response of memory B cells was not significantly affected by addition of anti-Ig, indicating that the stimulatory conditions provided by polyclonal activators were already optimal (Fig. 2B). In contrast, BCR triggering by anti-Ig was absolutely required by naïve B cells. Taken together, these results indicate that, in the absence of specific antigen, memory B cells proliferate and differentiate to antibody-secreting cells in response to polyclonal stimuli derived from microbes or activated T cells.

Continued indiscriminate stimulation of memory B cells by microbial products or bystander T cell help represents a plausible mechanism by which plasma cell generation and antibody production might be sustained in the absence of antigen throughout a human lifetime. In contrast to the current models, which evoke long-lived plasma cells or antigen persistence, the polyclonal activation model we present here makes three specific predictions. The first is that under steady-state conditions (i.e., in the absence of acute or chronic infection or recent vaccination), antigen-specific plasma cells should be continuously produced and should be detectable in peripheral blood, en route to the bone

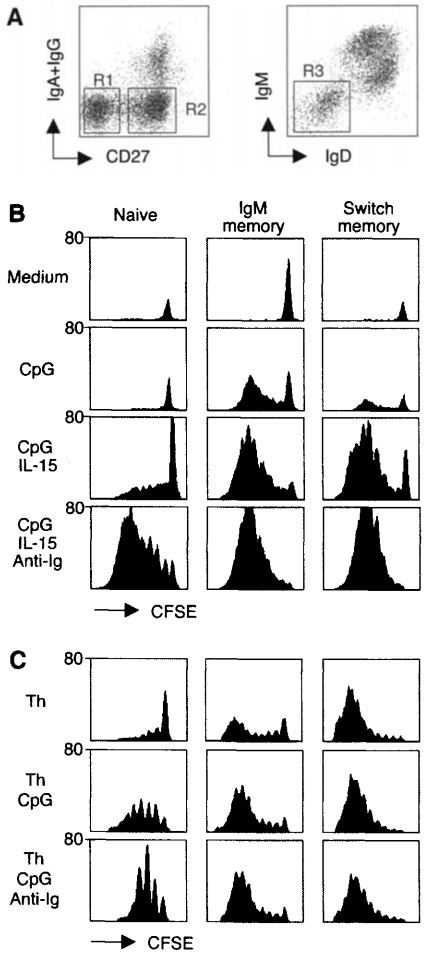

Fig. 1. Selective proliferation of human memory B lymphocyte subsets in response to CpG and bystander T cell help. (**A**) Gates used for sorting. $CD19^+$ cells isolated by magnetic sorting were stained with antibodies to CD27 and Ig isotypes (R1, naïve B cells; R2, IgM memory B cells; R3, switch memory B cells). (**B**) CpG stimulation. We cultured 3×10^4 CFSE-labeled B cells in the presence or absence of CpG 2006, IL-15, or $F(ab')_2$ fragments of goat antibody to human Ig. IL-15 and anti-Ig fragments alone or in combination failed to induce B cell proliferation. (**C**) Bystander T cell help (Th). We cultured 10^4 CFSE-labeled B cell subsets with an alloreactive T helper clone and stimulatory mononuclear cells (both irradiated) in the absence or presence of CpG 2006 or anti–human Ig fragments. Cell proliferation was measured on day 5. In all experiments, events were acquired for a fixed time so that the amplitude of the peak is proportional to the cell yield. Results are representative of at least 10 experiments. Comparable results were obtained using B cells isolated by negative selection.

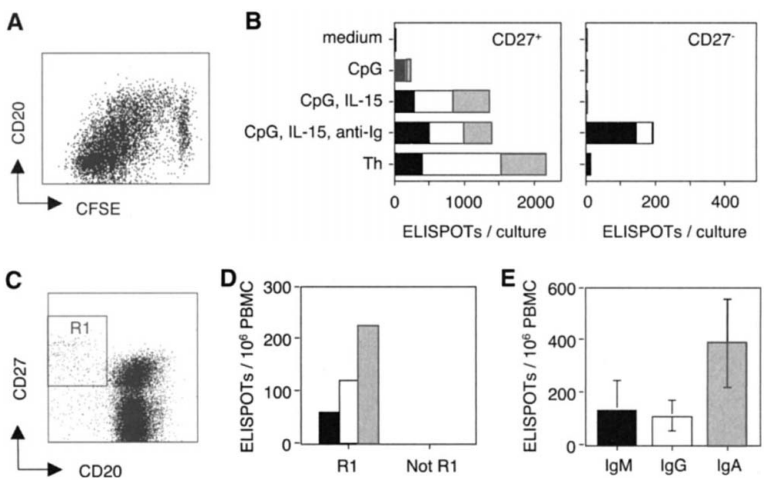

Fig. 2. (**A** and **B**) Differentiation of memory B cells to Ig-secreting cells. (A) CFSE-labeled memory B cells were stimulated for 5 days with CpG and IL-15, and the expression of CD20 was measured as a function of cell division. (B) Ig-secreting cells recovered on day 6 after stimulation of memory ($CD27^+$) or naïve ($CD27^-$) B cells. One representative experiment out of 10 is shown. (**C** to **E**) Circulating plasma cells. (C) Gate used for sorting and (D) Ig-secreting cells inside and outside the gate. (E) Circulating plasma cell levels (mean ± SD) in 20 healthy individuals. Ig-secreting cells were detected with isotype-specific ELISPOT. IgM, black bars; IgG, white bars; IgA, shaded bars [in (B), (D), and (E)].

marrow. Their frequency should reflect the frequency of antigen-specific memory B cells that respond to polyclonal activators. To test this, we used enzyme-linked immunospot (ELISPOT) assays to measure the frequency of circulating plasma cells secreting general antibodies (Ig) or antibodies to specific recall antigens (*14*). In peripheral blood of healthy donors, Ig-secreting cells were $CD27^{hi}$, $CD20^{-}$, $CD126^{+}$, and $CD38^{hi}$ (Fig. 2, C and D) (*15, 17*). Under steady-state conditions, the frequency of such cells was relatively constant (Fig. 2E), but strongly increased during ongoing infections or after vaccination (*15*). In one healthy donor tested 12 years after tetanus toxoid (TT) boost, the frequency of plasma cells secreting IgG antibodies to TT, relative to total IgG-secreting cells, was 1 in 1800 in peripheral blood and 1 in 950 in polyclonally stimulated cultures of memory B cells. In the same donor, serum anti-TT IgG was 5.1 μg/ml relative to 10 mg/ml total IgG (i.e., ~1 anti-TT molecule per 2000 IgG molecules). A similar correlation between circulating and in vitro–generated plasma cells was observed in several TT-immune donors (table S1). These findings indicate that under steady-state conditions, plasma cells specific for recall antigens are continuously generated at frequencies that are consistent with a polyclonal activation of memory B cells.

A second prediction of the polyclonal activation model is that under steady-state conditions (but not after boosting), the level of serum antibodies to a given antigen should reflect the frequency of specific memory B cells responsive to polyclonal activators. To test this prediction, we activated B cells with CpG and IL-15 and measured by limiting dilution the frequency of antigen-specific IgG memory B cells (*14*). In five donors examined several years after antigenic stimulation, there was a strong correlation between the frequency of IgG memory B cells specific for measles virus or TT and the respective serum antibody levels (Fig. 3, A and B). As expected, this correlation was lost after a TT boost, which led to an ~80-fold increase in serum antibody levels, accompanied by a much lower increase in B cell precursor frequency (*18*). We also compared IgG antibody titers in serum and supernatant of polyclonally activated B cells (Fig. 3C). In several donors and for most antibody specificities, the ratio of serum to supernatant fell within a half log distribution.

A third prediction of the polyclonal activation model is that during an antigen-specific response, the increased availability of activated T cells will lead to increased production of plasma cells of unrelated specificities. We therefore boosted five healthy donors with TT and measured, with time, the number of circulating plasma cells secreting IgG antibodies to TT or to other recall antigens. In a representative case (Fig. 4A), plasma cells secreting antibodies to TT (anti-TT) increased from day 6 to day 8 after boost and rapidly decreased over the next few days. IgG-secreting plasma cells increased with the same kinetics, and in all cases their number exceeded that of TT-specific plasma cells, consistent with an ongoing polyclonal activation. Indeed, the number of plasma cells secreting antibodies to unrelated antigens to which the donor was immune (*Toxoplasma gondii* and measles virus) also increased by a factor of ~10 with similar kinetics. These results indicate that (i) the antigen-dependent phase of plasma cell generation is limited to a brief window of time, and (ii) antigenic stimulation also leads to a polyclonal activation of memory B cells.

The boosting experiment also offered an insight into the relative role of short-lived and long-lived plasma cells. After TT boost, specific IgG antibodies increased by a factor of ~80 from day 5 to day 8, concomitant with the peak of plasma cell generation, and remained at a plateau level for 2 months, decreasing thereafter with a half-life of ~40 days (Fig. 4B). The kinetics of serum antibody levels can be explained by two distinct events: the rapid increase observed after boosting was due to the production of large numbers of short-lived plasma cells, whereas the plateau and the subsequent slow decline

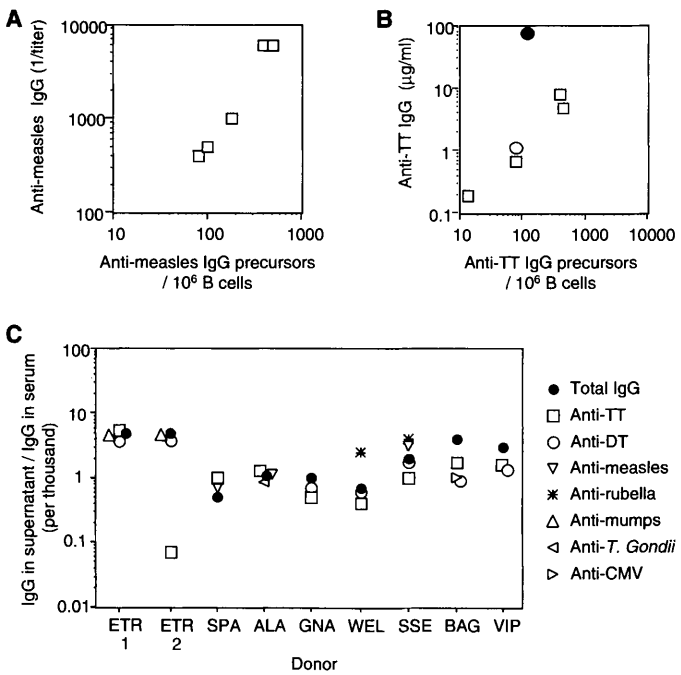

Fig. 3. (**A** and **B**) Correlation between serum antibody levels and frequency of memory B cells under steady-state conditions. The frequency of IgG memory B cells specific for measles virus or TT was measured by limiting dilution in the presence of CpG and IL-2. Serum antibody levels were determined by ELISA (enzyme-linked immunosorbent assay). Empty squares represent measurements taken from different donors with long-term immunity. In (**B**), the circles represent measurements taken in the same donor before (empty circle) and 4 weeks after (filled circle) TT boosting. (**C**) IgG antibody levels in the supernatant of polyclonally activated B cells relative to serum levels. One donor was tested before (ETR1) and 3 weeks after (ETR2) TT boost.

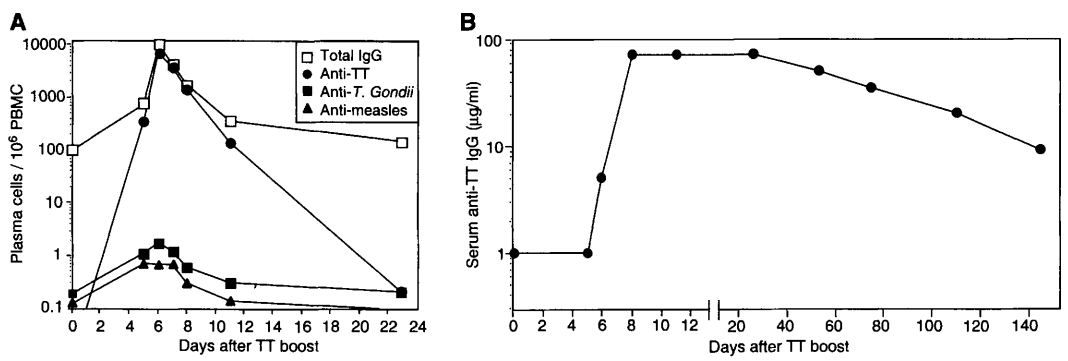

Fig. 4. (**A**) Kinetics of circulating plasma cells secreting IgG antibodies to TT or to unrelated antigens after a TT boost. A donor immune to TT, *T. gondii*, and measles virus was boosted with TT. At different times, the levels of circulating plasma cells secreting IgG (empty squares) and IgG antibodies to TT (filled circles), *T. gondii* (filled squares), or measles virus (filled triangles) were measured. (**B**) Time course of serum IgG antibodies to TT in the same donor. Comparable results were obtained in five donors.

in antibody levels [exceeding the ~20-day half-life of human IgG (*19*)] are consistent with the generation of long-lived plasma cells that turn over with a half-life of ~40 days.

Taken together, the above results indicate that memory B cells have two response modes. In the antigen-dependent mode, they undergo a massive expansion and differentiation toward short-lived plasma cells. This response is transient, because of the negative feedback exerted by the high level of antibody present (*20*). However, some plasma cells generated in this way become long-lived if rescued in available niches such as bone marrow (*21*). These cells sustain serum antibody levels, but can do so only for a few months, because of their limited life-span. In contrast, in the polyclonal mode, all memory B cells respond to environmental stimuli by undergoing continuous proliferation and differentiation. In this way, a constant level of plasma cells and serum antibodies could theoretically be maintained throughout a human life-span. Because this mechanism is nonspecific, it would act indiscriminately to maintain the broad spectrum of antibody specificities generated during the antigen-driven immune response.

The sensitivity to polyclonal stimuli represents a key feature of human memory B cells and adds a novel property to the concept of "memory stem cells" (*22, 23*). The differential response to polyclonal stimuli may reflect distinct roles of switch and IgM memory B cell subsets. Thus, although the exquisite sensitivity of switch memory B cells to bystander help may be instrumental in maintaining systemic IgG antibody levels, the capacity of IgM memory B cells to respond to CpG in the absence of cytokines could be instrumental in maintaining levels of natural antibodies to bacterial antigens (*24–26*). This may also underline the propensity to develop mucosa-associated lymphatic tissue (MALT) lymphomas under chronic infection by *Helicobacter pylori* (*27*). It is possible that polyclonal stimuli in addition to CpG or T cell help activate memory B cells in vivo within the microenvironment of secondary lymphoid organs or of the bone marrow, where memory B cells and proliferating plasma cell precursors are present (*28, 29*).

Our results discriminate between a "short-term serological memory," which is antigen-dependent and lasts for a few months, and a "long-term serological memory" that results from an antigen-independent polyclonal activation and differentiation of memory B cells. The possibility of selectively targeting one component or the other may open new ways for effective vaccination.

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Supporting Online Material

www.sciencemag.org/cgi/content/full/298/5601/2199/DC1
Materials and Methods
Table S1
References and Notes

12 July 2002; accepted 7 October 2002

Rainfall Variability, Carbon Cycling, and Plant Species Diversity in a Mesic Grassland

Alan K. Knapp,[1]* Philip A. Fay,[1] John M. Blair,[1] Scott L. Collins,[1,2] Melinda D. Smith,[3] Jonathan D. Carlisle,[1] Christopher W. Harper,[1] Brett T. Danner,[1] Michelle S. Lett,[1] James K. McCarron[1]

Ecosystem responses to increased variability in rainfall, a prediction of general circulation models, were assessed in native grassland by reducing storm frequency and increasing rainfall quantity per storm during a 4-year experiment. More extreme rainfall patterns, without concurrent changes in total rainfall quantity, increased temporal variability in soil moisture and plant species diversity. However, carbon cycling processes such as soil CO_2 flux, CO_2 uptake by the dominant grasses, and aboveground net primary productivity (ANPP) were reduced, and ANPP was more responsive to soil moisture variability than to mean soil water content. Our results show that projected increases in rainfall variability can rapidly alter key carbon cycling processes and plant community composition, independent of changes in total precipitation.

Anthropogenic climate change is projected to include increasingly variable precipitation regimes, as well as atmospheric warming (*1*). General circulation models forecast a higher frequency of extreme rainfall events from intense convective storms, a lower frequency of rainfall days, and longer intervening dry periods (*1–5*). Evidence is mounting that an increase in precipitation extremes has begun to occur worldwide (*5–8*). Most aspects of terrestrial ecosystem structure and function are vulnerable to these hydrologic changes, perhaps independent of changes in annual precipitation quantity (*1, 9, 10*), and important interactions with elevated temperatures and atmospheric carbon dioxide can be expected (*11*). Thus, models and policy analyses of the consequences of climate change should not rely on scenarios that focus primarily on climatic means (*12, 13*). Yet, our ability to forecast ecosystem responses to climate change is constrained by a lack of

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