uses the 20% bacterial growth efficiency that we report[1]. We derived this efficiency precisely from the respiration measurements which Geider criticizes in his comment.

If one accepts this 20% growth efficiency, as many do[2–5], then bacterial respiration is four times larger than bacterial production. Let us combine, then, this value of bacterial growth efficiency with published data on bacterial production and primary production for the world's oceans to derive an independent figure for bacterial respiration. The most comprehensive review is that of Ducklow and Carlson[6]. Using these data, the resulting bacterial respiration represents on average 102 and 110% of net primary production in open ocean and coastal marine systems, respectively. Stated more broadly, if bacterial production is ~30% of net primary production, as has been widely reported[7], when bacterial growth efficiency is 23% or lower, bacterial respiration will exceed primary production. Possibly net heterotrophy is more widespread in the oceans than is currently accepted, or bacterial growth efficiency is much higher than 20%, or most measurements of bacterial production are serious overestimates, relative to primary production.

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Lifetime of plasma cells in the bone marrow

Immune protection is based on long-lived memory cells and effector cells, which are either cytotoxic or secrete antibodies. The lifespan of these effector cells has not so far been determined. Here we show that antibody-secreting plasma cells from bone marrow are as long-lived as memory B cells.

After immunization with T-cell-dependent antigens, specific antibody titres are often stable for long periods. As the half-life of antibodies is short[1], the observed antibody titre must be maintained by antibody-secreting plasma cells. In the bone marrow, antigen-specific plasma cells can be detected for at least one year after immunization[2], but whether these plasma cells are continuously generated by B cells that are activated by persisting antigens, or whether they are long-lived, is not known[3]. Analysis of proliferation of total plasma cells in bone marrow over a 10-day period has suggested that most plasma cells may have a lifespan of only a few weeks[4]. In splenic foci, plasma cells that are derived from B cells after primary immunization die by apoptosis after a few days[5].

We investigated the lifespan of murine bone-marrow plasma cells in a secondary immune response to ovalbumin. We used cellular-affinity matrix technology[6] to identify, isolate and analyse IgG1 antibody-secreting cells (Fig. 1a). Such cells secrete ovalbumin-specific IgG1 antibodies when isolated and cultured (about 0.3–0.5 ng per cell per day; data not shown), and could be identified by intracellular binding of ovalbumin and low expression of surface immunoglobulins.

After secondary immunization with ovalbumin, the absolute number of these plasma cells in the bone marrow (about 5×10^{-4}, ~75,000 per mouse), as determined by flow cytometry, and the specific antibody titre (>1 mg per ml serum) remain constant for more than 3 months, whereas in the spleen, the number of plasma cells drops from a peak of 150,000 per

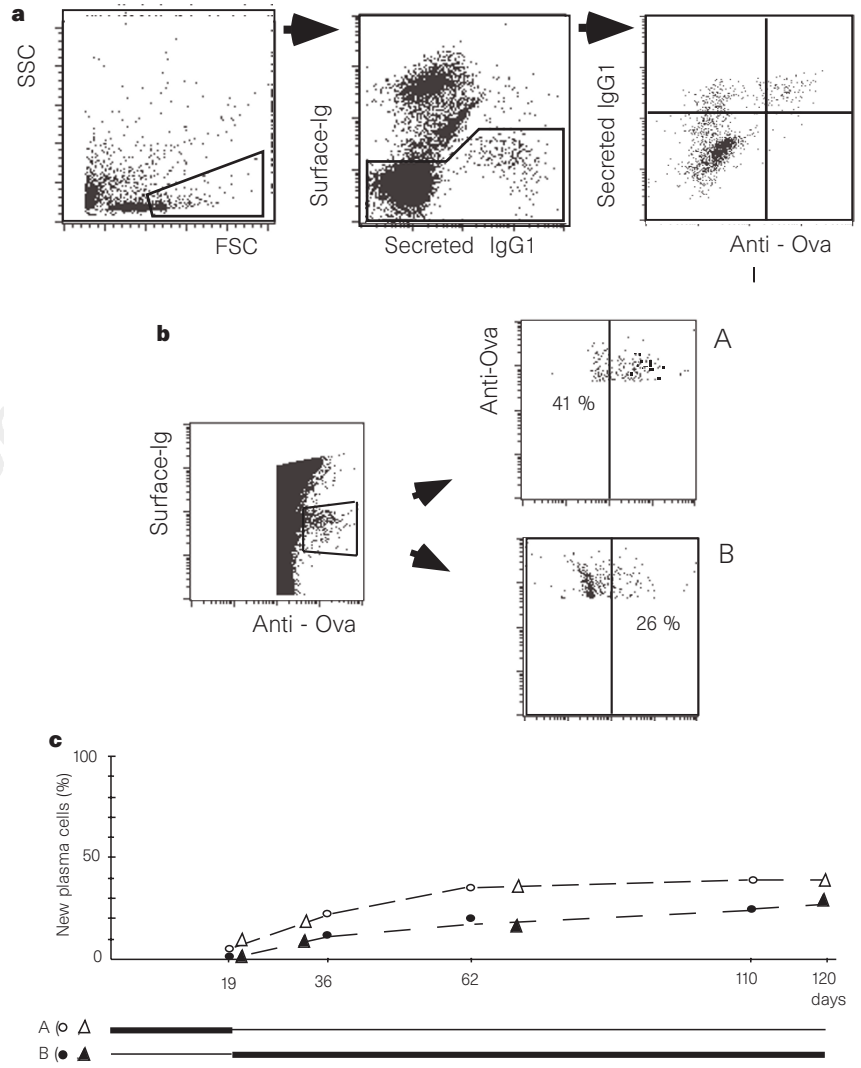

Figure 1 Lifetime analysis of antibody-secreting plasma cells. BALB/c mice were immunized with 0.1 mg alum-precipitated ovalbumin (Ova), and boosted 32 days later with 0.1 mg soluble ovalbumin. Femoral bone marrow of three mice was pooled for each stage of the analysis. **a,** Identification of IgG1-secreting, ovalbumin-specific plasma cells in the bone marrow as blast (forward scatter/side scatter, FSC/SSC), low surface immunoglobulin, high secreted IgG1, intracellular anti-ovalbumin-positive cells, 12 days after boost, with analysis gates as indicated. **b,** Analysis of BrdU incorporation as a measure of DNA synthesis by these bone-marrow plasma cells, 110 days after secondary immunization. BrdU incorporation was determined for group A and B plasma cells (see **c**), by staining with fluorescein-labelled anti-BrdU, with evaluation gates set according to controls (not shown). **c,** Replacement kinetics of ovalbumin-specific bone-marrow plasma cells after ovalbumin boost (day 0). BrdU feeding periods (1 mg per ml drinking water) are indicated by thickened bars. Frequencies of BrdU-negative (A) and BrdU-positive (B) bone-marrow plasma cells. Two experiments are indicated by circles and triangles. (Details of methodology are available from the authors on request).

mouse at day 6 to below 10,000 per mouse from day 11 onwards (data not shown). Therefore, just two weeks after secondary immunization, the majority of specific plasma cells reside in the bone marrow, as expected[7].

We determined the life-span of ovalbumin-specific plasma cells residing in the bone marrow using bromodeoxyuridine (BrdU) incorporation as a measure for DNA synthesis, a method that had already been used successfully to measure the life-span of memory B cells[8]. We immunized and boosted two groups of mice with ovalbumin. Group A was given BrdU in drinking water for the first 19 days after secondary immunization. For group B, feeding with BrdU started at day 19 after the secondary immunization and was maintained for more than 90 days. We measured BrdU incorporation by ovalbumin-specific plasma cells from each group at various times (Fig. 1b, c). Nearly all of the specific plasma cells developed within two months of secondary immunization. Later, only very few additional plasma cells were found to be labelled in group B, or unlabelled in group A. Group A consistently shows 5–15% more 'new' plasma cells, which probably represent the unlabelled plasma cells from the primary immunization, so they are really very 'old' plasma cells. Apparently, these plasma cells had not proliferated on secondary immunization with ovalbumin. Between primary and secondary immunization, the specific serum titre increased by 10–40 times, corresponding to the 20-fold increase in bone-marrow plasma cells.

To conclude, more than 60% of the plasma cells at day 110 after the booster injection lived for more than 90 days without DNA synthesis. They may live longer, as suggested by the asymptotic slope of the incorporation curve. Our data provide clear evidence that most plasma cells, present in constant numbers in the bone marrow, are long-lived and not derived from the differentiation of proliferating, activated B cells. These plasma cells may be as long-lived as memory B cells. Our result has implications for the design of vaccination strategies and may aid the understanding of allergy and humoral autoimmunity.

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Importance of ancestral DNA ages

Recent publications consider the age of a common ancestor of samples of human DNA sequences[1–9]. In particular, variation in Y chromosomes has been interpreted by different authors to give very different estimates of the time to the most recent common ancestor of the sample[1–8]. The cause of the differences is that the data are being interpreted by various authors in terms of their preferred models of human demography. The data are so lacking in power that these estimates depend hardly at all on the data, and almost entirely on the demographic model assumed. However, if we knew the demography of early humans, we would have no real interest in the time to the most recent common ancestor of an individual gene, as the time is important only because it tells us about the demography.

Generally, the data are a sample of sequences, D. Assumptions about the neutral mutation rate, μ, and a demographic model, N (often including neutrality and a constant effective population size) are used to calculate $P(T|D,N,\mu)$, the probability that the sequences had a time to most recent common ancestry of T, conditional upon D, N and μ. From these, one can calculate an expected value of T, $E(T)$.

Different values of $E(T)$ originate from differing choices of N. A clear example concerns analyses of the data of Dorit *et al.*[1], who found no variation in a sample of 38 Y chromosomes for a 729-base-pair intron from the *ZFY* gene. They estimated $E(T)$ using two models. One incorporated a constant population size, with all values of T equally likely prior to the data. Another model assumed a population expansion again with a constant prior distribution of T. $E(T)$ values were 270,000 and 27,000 years, respectively. Others sought explicitly to condition on the basis of a demographic model. Fu and Li[2] considered a constant population size model, and, depending upon the effective population size (from 2,500 to 30,000) obtained values for $E(T)$ ranging from 92,000 years to 703,000 years. Donnelly *et al.*[3] assumed a lognormal prior distribution of population size with a mean of 36,000. $E(T)$ values ranged from 254,000 to 460,000 years, depending on μ. Weiss and von Haeseler[4] imagined populations exponentially increasing, with various growth rates, creating a range of $E(T)$ values from 17,000 to 286,000 years.

The explanation of this 40-fold range of expected times is that, when data sets lack power, $E(T)$ depends more on N than on D. An extreme example is the analysis by Fu and Li[5] of the data of Knight *et al.*[9]. The sequence diversity of an Alu insertion at the α-globin-2 locus suggested an $E(T)$ of under 100,000 years. In their re-analysis, Fu and Li calculate $E(T)$ values over 450,000 years. They use a neutral model with a constant 10,000 individuals, such that $E(T|N) \approx 800{,}000$ years, prior to the data, the lack of power of which leaves $E(T|D,N,\mu)$ high.

The demographic models are assumed to be known without error, and are usually not tested for consistency with the data. Yet T is interesting only for the information that it supplies about N, the demography of the human species. It is N, not T, that can be related to the fossil record, for example. We wish to know human population sizes in the past, and to investigate the possibility of demographic expansions during the recent spread from Africa. Values for times to common ancestry calculated on the basis of an assumption that there has been no expansion are merely confusing.

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correction

In "Structures of mollusc shell framework proteins", by S. Sudo *et al.* (*Nature* **387,** 563–564; 1997) the deduced amino-acid sequence of pMSI2 (Fig. 1b) contained an error at position 69. The corrected sequence is reproduced below.

b

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MKPFVTLASLIVLIASASADGYDDYKKYGSVGYGPGISLGGGGLGGGGGIISVGGGGGLGGGLGGGLGCG   70
LVGVGGGGLIGGGFGPGRVSGTINAGGGVFASGSGLGGGLSPAGRGAAQGAATLSALQIASGRPGRVSGVS  140
VGTGGGRAVVSGSATPVGGFGVPYGGYGYNYGVPSYGVGLPSYGVSLPSYGVGLGGGYGGYGYGLDLASF  210
QGSTYGNLATGQINTAVVAFHMAVLLESMEAESDTEVDTEDMDSEEDMESEEDTESEEDTESEEDTESEE  280
DMESEEDMDSESSVVDQVSMVYPNHFTGDVLFCQVRLQELEFPALEALVSVVLE                   334
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