tsunamis from great earthquakes in the Cascadia subduction zone.

3) Tectonic subsidence during great subduction earthquakes could reconcile rates of short-term uplift with rates of long-term uplift in westernmost Washington. The uplift measured at tide gages and bench marks (2 to 3 mm per year average during the past 50 years) is much faster than that inferred from Pleistocene marine terraces (<0.5 mm per year average during the past ~100,000 years) (*18*). But these rates need not conflict if, as part of cyclic earthquake-related deformation (*19*), coseismic subsidence (like that inferred from the buried lowlands) has nearly negated cumulative interseismic uplift (of which tide-gage and bench-mark uplift would be a modern sample).

Jerky Holocene submergence at Washington estuaries thus strengthens the hypothesis that a future great earthquake could emanate from the Cascadia subduction zone. The number and shallow depth of buried lowlands at Willapa Bay (Fig. 3C) may mean that at least six such earthquakes have occurred since sea level approached its present position on mid-latitude coasts, that is, since 7000 years ago (*20*). The earthquake ruptures, if really from events of magnitude 8 or greater, should have extended coastwise for at least 100 km (*21*). This corollary can be tested by determining the coastwise extent of individual episodes of coseismic subsidence. Another testable corollary is that shaking during the postulated earthquakes should have caused the liquefaction of Holocene coastal-lowland sand (*22*). If buried lowlands prove coeval for coastwise distances greater than 100 km, and if sand proves to have vented onto some of these lowlands at the start of burial, then the chronology of jerky submergence could be used to constrain the current probability of a great subduction earthquake in the Pacific Northwest.

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3. Episodic late Cenozoic thrusting in the Cascadia subduction zone is implied by faults, folds, and unconformities in the overlying North America plate. Unless accomplished by episodic creep, such thrusting would suggest that great Cascadia earthquakes have occurred in the Quaternary [P. D. Snavely, Jr., in *Am. Assoc. Petrol. Geol. Mem.*, D. W. Scholl, A. Grantz, J. G. Vedder, Eds., in press].
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Interferon-γ and B Cell Stimulatory Factor–1 Reciprocally Regulate Ig Isotype Production

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Gamma interferon (IFN-γ) and B cell stimulatory factor–1 (BSF-1), also known as interleukin-4, are T cell–derived lymphokines that have potent effects on B cell proliferation and differentiation. They are often secreted by distinct T cell clones. It is now shown that IFN-γ stimulates the expression of immunoglobulin (Ig) of the IgG2a isotype and inhibits the production of IgG3, IgG1, IgG2b, and IgE. By contrast, BSF-1 has powerful effects in promoting switching to the expression of IgG1 and IgE but markedly inhibits IgM, IgG3, IgG2a, and IgG2b. These results indicate that BSF-1 and IFN-γ as well as the T cells that produce them may act as reciprocal regulatory agents in the determination of Ig isotype responses. The effects of IFN-γ and BSF-1 on isotype expression are independent.

GAMMA INTERFERON (IFN-γ) promotes the production of immunoglobulin (Ig) by activated murine and human B cells stimulated with interleukin-2 (*1*) and causes human B cells treated with antibodies to Ig to enter the S phase of the cell cycle (*2*). Conversely, IFN-γ inhibits the actions of B cell stimulatory factor–1 (BSF-1) on resting B cells, including BSF-1 induction of class II major histocompatibility complex molecule expression (*3*) and costimulation of proliferation (*4*). IFN-γ also suppresses the enhancement by BSF-1 of IgG1 and IgE synthesis in B cells stimulated with lipopolysaccharide (LPS) (*5*). We show here that IFN-γ induces a selective and striking induction of IgG2a production by resting B cells stimulated with LPS in vitro. Furthermore, both IFN-γ and BSF-1 are potent inhibitors of the expression of specific Ig isotypes; IFN-γ blocks IgG3 and IgG2b (*6*) as well as IgG1 and IgE, whereas BSF-1 blocks IgG3, IgG2b (*7*), IgG2a, and IgM. These results suggest that IFN-γ and BSF-1 reciprocally regulate Ig isotype production in T cell–dependent immune responses and thus determine many of the biologic consequences of such antibody production. Since BSF-1 and IFN-γ appear to be produced by separate sets of T cell clones (*8*), a reciprocal regulatory interaction of T cell subsets may determine Ig isotypic responses to immunization.

Resting B cells were purified from spleens of 8- to 12-week-old DBA/2 mice by incubation with antibodies to Lyt-1, Lyt-2, and Thy 1.2 and complement, followed by Percoll density-gradient centrifugation (*4*). When these cells were stimulated with LPS, they synthesized large amounts of IgM, considerable IgG3 and IgG2b, and small but detectable amounts of IgG1 and IgG2a (*7*). Addition of recombinant IFN-γ (rIFN-γ) (10 U/ml) (*9*) caused a striking increase in IgG2a concentrations and near complete suppression of IgG3, IgG1, and IgG2b production, but had little effect on IgM (Fig. 1). At concentrations of rIFN-γ 30 to 100 times the amount needed to inhibit IgG3, IgG1, and IgG2b completely, suppression of both IgM and IgG2a occurred and could be explained in large part by the striking diminution in viable cell yields at these high rIFN-γ concentrations.

Addition of a hamster monoclonal anti-

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body to rIFN-γ (*10*) completely reversed both the enhancing and suppressive effects of rIFN-γ on isotypes of Ig secreted by LPS-stimulated B cells, clearly demonstrating that these effects are due to IFN-γ itself and not to some possible contaminant in the rIFN-γ preparation (Fig. 2). The antibody to rIFN-γ had no effect on the concentrations of secreted isotypes of B cells treated with LPS only and by itself did not score in the enzyme-linked immunosorbent assay (ELISA).

The effect of rIFN-γ in enhancing IgG2a production could be achieved by treatment of resting B cells before the addition of LPS. Resting B cells incubated for 48 hours in the presence or absence of rIFN-γ (20 U/ml) were rigorously washed, and equal numbers of viable cells were cultured in the presence of LPS or LPS and antibody to rIFN-γ. Cells incubated in rIFN-γ showed a tenfold increase in the concentration of secreted IgG2a compared to cells incubated in medium alone (Table 1). These cells also showed a three- to fourfold suppression of IgG3. Cells treated with rIFN-γ showed only a modest increase in viable cell yields compared to the group treated with medium, too small an increase to explain the striking increase in IgG2a. Similar results were obtained in the presence or absence of antibody to rIFN-γ in the "secondary" cultures, indicating that rIFN-γ was not significantly carried over into these cultures after washing. We showed earlier that BSF-1 can act on resting B cells to prepare them to secrete IgG1 upon subsequent stimulation with LPS (*11*). Therefore, both IFN-γ and BSF-1 can act on the resting B cell to specifically regulate Ig isotype secretion upon subsequent addition of LPS.

BSF-1 can cause a 90 to 95% inhibition in the production of IgM, IgG3, IgG2b, and IgG2a in response to LPS, with only modest diminution in cell yield (*7, 12*). The capacity of rIFN-γ to enhance IgG2a production appears to be independent of this inhibitory effect of BSF-1, since the relative rIFN-γ–induced increase in IgG2a concentrations and the rIFN-γ concentration required for induction are the same over a range of recombinant BSF-1 (rBSF-1) (*13*) concentrations that progressively inhibit IgG2a secretion (Fig. 3).

The inhibitory effects of rIFN-γ on IgE and IgG1 also appear to be independent of the stimulatory actions of rBSF-1 (Fig. 4). Thus, the concentrations of rIFN-γ required to inhibit IgE and IgG1 are the same for a wide range of rBSF-1 concentrations. In particular, IgE concentrations can be moderately or strikingly enhanced by rBSF-1, at 600 or 10,000 U/ml, respectively, and IgG1 levels show a bimodal stimulatory response

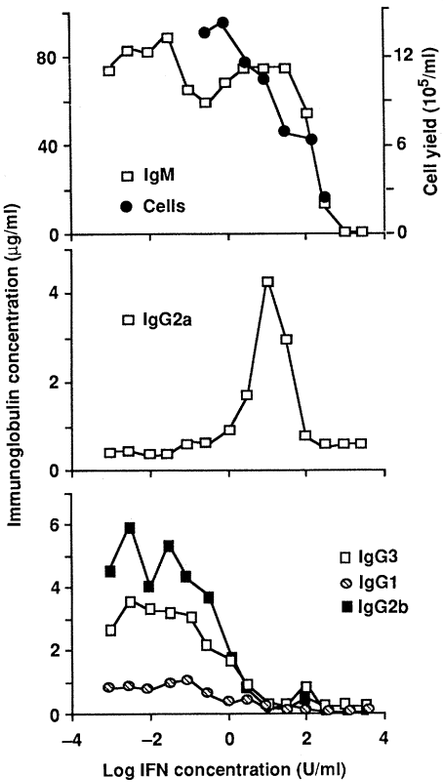

Fig. 1. IFN-γ modulates Ig isotype secretion by LPS-activated B cells. Purified resting B cells were obtained by discontinuous Percoll gradient centrifugation and were distributed into 96-well microtiter plates at 1×10^4 cells in 200 μl of culture medium [RPMI 1640, 10% fetal bovine serum, L-glutamine (2 m*M*), 2-mercaptoethanol (0.05 m*M*), penicillin (50 μg/ml), and streptomycin (50 μg/ml)]. Cells were then stimulated with LPS (20 μg/ml) and increasing concentrations of murine recombinant IFN-γ prepared in Chinese hamster ovary cells. Cultures were carried for 6 days at 37°C in a 6% CO_2 atmosphere, after which culture supernatants were removed for analysis of Ig isotypes by a solid-phase immunoassay (ELISA) (*12*) and for determination of viable cell yield. The ELISA assay, in which a fluorescent product was generated by specifically bound alkaline phosphatase–conjugated antibodies acting on the substrate methyl umbelliferyl phosphate, specifically detected individual Ig isotypes even when high concentrations of other Ig isotypes were present in the culture supernatants. Cultures were established in duplicate for each point. Results are reported as the mean Ig isotype concentration. For the data points presented in Figs. 1 to 4, the mean relative standard error (± SD) is 0.113 ± 0.089.

to rBSF-1, with peaks at 125 and 10,000 U/ml. The latter effect is highly reproducible in detailed concentration-response titration and will be reported separately (*12*). The inhibitory effects of rIFN-γ are essentially the same in each of these groups and cannot be explained by diminution of cell yield, since IgG1 and IgE production are almost completely inhibited by rIFN-γ at 10 U/ml, whereas >100 U/ml is required for comparable inhibition of cell yields (Fig. 1).

Further support for the independence of action of rBSF-1 and rIFN-γ on switching to IgG1 production is shown by the finding that rIFN-γ can fully inhibit IgG1 production when added after rBSF-1 has been removed from culture. In these experiments, B cells were incubated with medium or rBSF-1 for 48 hours, washed, and stimulated with LPS with or without rIFN-γ. Cells treated with rBSF-1 produced 6.5 μg per milliliter of IgG1 on subsequent stimulation with LPS, a 12-fold enhancement in IgG1 secretion over those treated in medium only. Addition of rIFN-γ and LPS at the same time reduced IgG1 production to 0.3 μg/ml in the group treated with BSF-1. The concentrations of rIFN-γ used (20 U/ml) did not diminish IgM production, an indication that this "late" inhibitory effect of rIFN-γ on IgG1 production could not be explained by inhibition of cell growth.

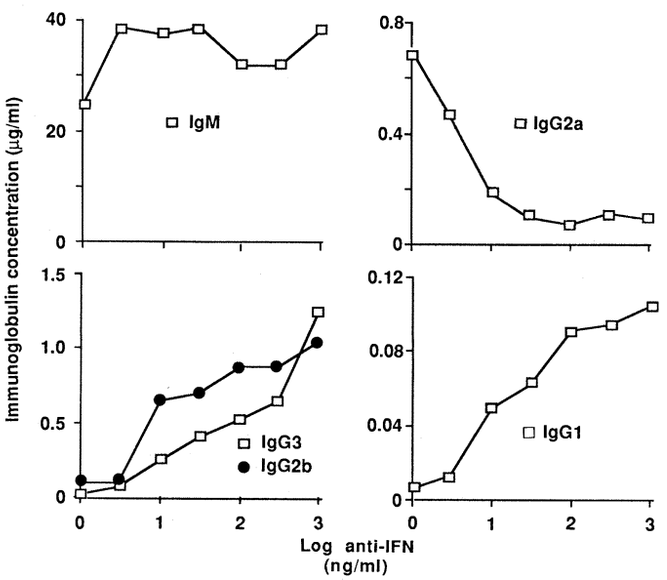

Fig. 2. Antibody to IFN-γ (anti–IFN-γ) reverses the effect of IFN-γ on LPS-stimulated murine B cells. Purified resting B cells were stimulated with LPS (20 μg/ml) and recombinant IFN-γ (20 U/ml) in the presence of increasing concentrations of purified monoclonal anti-IFN-γ (*10*). Culture supernatants were removed after 6 days for measurement of Ig isotype concentrations by ELISA.

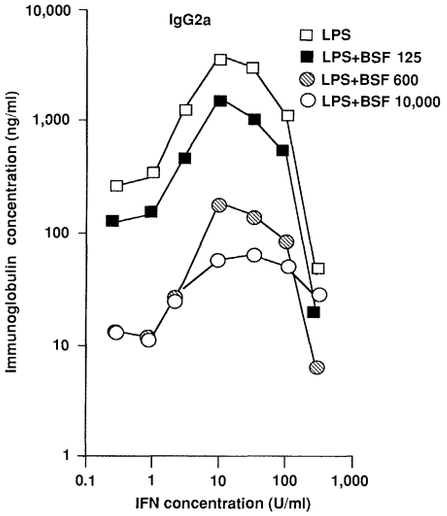

Fig. 3. IFN-γ enhancement of IgG2a secretion is independent of BSF-1 action. Purified resting B cells were stimulated with LPS (20 μg/ml), recombinant BSF-1 at 0, 125, 600, and 10,000 U/ml, and increasing concentrations of recombinant IFN-γ. Ten units of rBSF-1 is approximately equal in activity to 1 U of T cell–derived BSF-1, measured as described by Ohara *et al.* (*13*). Culture supernatants were removed after 6 days for measurement of IgG2a concentrations by ELISA.

Our results indicate that both rIFN-γ and rBSF-1 promote the expression of specific Ig isotypes while inhibiting the expression of other isotypes. Recent studies suggest these reciprocal regulatory effects may also operate in vivo. Antibody to BSF-1 blocks IgE production by helminth-infected mice (*14*) and IFN-γ enhances serum IgG2a concentration in mice treated with antibody to IgD (*15*). Although the mechanism through which IFN-γ exerts its stimulatory effects on IgG2a production has not been established, it seems likely that it, like BSF-1, acts to cause Ig class switching. BSF-1 added prior to (*13*) or together with LPS (*7*) enhances IgG1 production by B cells selected for the absence of membrane IgG expression.

The demonstration that the production of IFN-γ and of BSF-1 are largely segregated in cloned T cell lines (*8*) suggests that the distinctive Ig isotype regulatory patterns of these lymphokines will also be true of the cells that secrete them. It has been proposed that IFN-γ–producing T cells be designated T_H1 cells; these cells also secrete interleukin-2. By contrast T cell clones that secrete BSF-1 have been designated T_H2 cells; these cells fail to produce either IFN-γ or interleukin-2.

The effect of IFN-γ on IgG2a production is particularly interesting in view of the capacity of IFN-γ to enhance the expression of human Fc receptors analogous to the mouse FcR1 receptor specific for IgG2a (*16*). IgG2a antibody is the most effective isotype for the induction of macrophage and killer cell antibody-dependent cellular cytotoxicity (ADCC) of tumor cells, whereas IgG1 has very limited activity in ADCC (*17*). Furthermore, the marked superiority of IgG2a over IgG1 in complement fixation (*18*) indicates the striking biologic difference in antibodies of these two isotypes. These findings suggest that IFN-γ may be important in immune responses in which ADCC, opsonization, and complement-mediated lysis play an important protective role. In this regard, it is striking that IgG2a is the predominant IgG isotype in antibody responses to a series of DNA and RNA viruses, in which opsonization and complement-mediated lysis of viruses and destruction of virus-infected cells by ADCC may be of great importance (*19*). Indeed, the protective effects of antibody in herpes simplex virus infections and its immunotherapeutic role in Friend leukemia virus–induced diseases depend on intact Fc portions (*20*).

This leads us to propose an evolutionary selection for the production of the IgG isotype most protective against such infections—namely, IgG2a—through the preferential activation of the set of T cells that secrete IFN-γ (T_H1 cells). Although it is beyond the scope of this discussion, we wish to point out that BSF-1 is important in the regulation of IgE production (*14*), that it induces the expression of IgE-binding Fc receptors on B cells (*21*) and enhances the growth of mast cells (*22*). This suggests that BSF-1 and T_H2 cells may be adapted to responses to certain parasitic agents. The determination of the relative sensitization of T_H1 and T_H2 cells in response to viral infections and to other immunogens will be of critical importance in testing this concept. If T cell subsets have distinctive response patterns, the determination of the properties that cause the activation of cells of these two subsets will be of critical importance in clarifying the regulation of Ig isotype production.

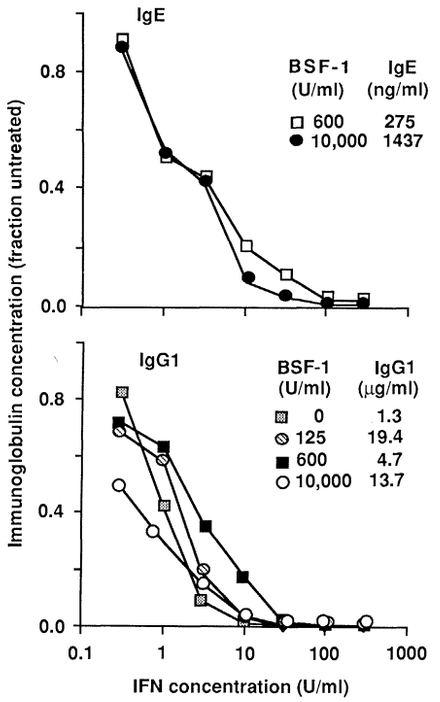

Fig. 4. IFN-γ inhibition of LPS-induced secretion of IgG1 and IgE is independent of BSF-1 action. Purified resting B cells were stimulated with LPS (20 μg/ml), recombinant BSF-1 (0, 125, 600, and 10,000 U/ml), and increasing concentrations of recombinant IFN-γ. Culture supernatants were removed after 6 days for measurement of IgE and IgG1 concentrations by ELISA. Ig isotype levels are expressed as a fraction of levels obtained in the absence of IFN-γ.

Table 1. IFN-γ acts on resting B cells to modulate Ig isotype secretion upon subsequent stimulation with LPS. Small resting B cells were preincubated in medium with or without IFN-γ (20 U/ml) for 48 hours. Cells were washed three times and stimulated with LPS (20 μg/ml) or LPS and anti–IFN-γ (2 μg/ml) for an additional 6 days. Culture supernatants were removed for measurement of Ig isotype concentrations by ELISA. Each group consists of three replicates. Values reported are mean Ig concentrations; relative standard errors varied between 4% and 17%.

Preincubation	"Secondary" cultures	IgG3 (ng/ml)	IgG2a (ng/ml)	Viable cell yield (10^5/ml)
Medium	LPS	875	24	11.2
	LPS + anti–IFN-γ	587	27	12.0
IFN-γ	LPS	230	250	14.2
	LPS + anti–IFN-γ	215	287	14.0

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Electrophoretic Evidence for Genetic Diploidy in the Bracken Fern (*Pteridium aquilinum*)

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Analysis of isozyme variability demonstrates that bracken fern (*Pteridium aquilinum*) has a diploid genetic system and expresses solely disomic inheritance patterns. Electrophoretic data indicate that genetically variable progeny are produced in natural populations after intergametophytic mating rather than by a process involving recombination between duplicated unlinked loci. Although some enzymes are encoded by more than one locus, this has resulted from subcellular compartmentalization of isozymes, and there is no evidence of extensive gene duplication resulting from polyploidy. The conclusions reached in this report differ from those which propose polyploidy as an adaptive mechanism for maintaining genetic variability in *Pteridium* and other homosporous pteridophytes.

THERE ARE TWO OPPOSING THEORIES to explain the production of genetically variable offspring in homosporous pteridophytes. The more conventional theory proposes that outcrossing generates heterozygosity and subsequent meiotic segregation leads to variable progeny (*1–4*). However, the unique life-cycle characteristics of homosporous pteridophytes inspired Klekowski and Baker (*5*) to formulate an alternative theory concerning the genetic behavior of these plants. The homosporous pteridophytes differ from other vascular land plants in producing wholly independent, potentially bisexual gametophytes. If these gametophytes self-fertilize, they give rise to sporophytes that are homozygous at all genetic loci. The potential for this reproductive process led to the proposal that homosporous pteridophytes are primarily inbreeding (*5*). A second distinctive feature of homosporous pteridophytes is that they typically have high chromosome numbers, suggesting that they are highly polyploid. According to Klekowski and Baker (*5*), such polyploidy could be a genetic adaptation required to overcome the extreme homozygosity imposed by recurrent inbreeding. To release variability from these homologously homozygous plants, Klekowski proposed that homoeologous chromosomes (those from different genomes within a polyploid) pair during meiosis (*6*). Such pairing would result in recombination between different genomes and the subsequent release of genetic variability among progeny.

Several approaches, including cytological studies (*7*), segregation of morphological markers (*8*), and segregation of electrophoretically detectable genetic markers (*9*), have been used to detect the results of homoeologous pairing. However, more recently, several studies have questioned the universality of Klekowski's hypotheses by demonstrating that some homosporous pteridophytes are outcrossing (*2–4*) and genetically diploid (*10, 11*). These studies, however, did not include the species that had been used to demonstrate homoeologous pairing. By means of electrophoretic analysis of enzyme variability, Chapman *et al.* (*9*) found that in bracken fern (*Pteridium aquilinum*) several enzymes were expressed as multiple bands. Chapman *et al.* (*9*) interpreted these results as evidence that there were duplications in the coding genes resulting from polyploidy. Furthermore, the variability expressed among siblings was attributed to homoeologous recombination in the parental generations.

Although these results seemed conclusive at the time, it has been shown that multiple-banded isozyme patterns do not always indicate polyploidy. Use of an inappropriate grinding buffer during preparation can lead to enzyme breakdown and subsequent ghost banding or poor band resolution (*12*). Multiple bands can also result from assaying enzymes composed of more than one subunit. For example, a dimeric enzyme appears as one band in homozygotes but as three bands in heterozygotes (*13*). Also, many diploid plants have been shown to express several isozymes for certain enzymes, each isozyme encoded by a separate genetic locus within a single genome (*14*). These isozymes are active in different compartments (for example, chloroplasts or cytosol) within the cell (*15*). Multimeric isozymes and enzyme compartmentalization were not discussed by Chapman *et al.*, and the grinding buffer they used was a simple one (*9*), which may have resulted in enzyme breakdown.

The present study reassesses the inheritance patterns of polymorphic structural genes in *P. aquilinum* by means of updated protocols for horizontal starch gel electrophoresis (*16*). This study also differs from that of Chapman *et al.* (*9*) in that we directly analyzed gametophytic progeny (representing individual meiotic products), rather than examining sporophytes arising from self-fertilized gametophytes. Thirty-nine single-frond spore samples were collected from wild sporophytes across the United States, Mexico, and Europe (*17*). Each spore sample was sown separately onto nutrient agar medium (*18*) and cultured under standard conditions (*19*). When gametophytes were 3 weeks old, prior to maturation of gametangia, they were harvested for electrophoretic analysis. At least ten gametophytes from each spore sample (giving a total of

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