Tsujimoto, C. M. Croce, *Cell* **49**, 455 (1987)] with either a PGK-*neo*-polyadenylate [poly(A)] cassette derived from pKJ-1 [V. L. Tybulewicz, C. E. Crawford, P. K. Jackson, R. T. Bronson, R. C. Mulligan, *Cell* **65**, 1153 (1991)] or a PGK-hyg-poly(A) cassette (*11*), respectively. The targeting vectors contained 6.5 kb of homology 5′ and 1.1 kb 3′ of the drug-resistance marker. The PGK-*tk*-poly(A) cassette (V. L. Tybulewicz *et al.*, as above) was ligated into a restriction site in a vector polylinker at the 5′ end of the insert. E14 and D3 ES cell lines were maintained as described [E. J. Robertson, *Embryo-Derived Stem Cell Lines: Teratocarcinomas and Embryonic Stem Cells: A Practical Approach*, E. J. Robertson Ed. (IRL Press, Oxford, 1987)]. A transfection was performed using pbKO-NEO, then homologous recombinants were further transfected with pbKO-HYG. G418 selection (300 μg/ml; Gibco BRL, Gaithersburg, MD) and gancyclovir selection (2 μM) at the first-round transfection, or G418, hygromycin B (150 μg/ml; Sigma, St. Louis, MO), and gancyclovir selection at the second-round transfection was started 24 hours later. Cell lysate of the clones was used as a template for PCR amplifications with a *bcl-2* flanking primer (5′-ATTCGTTCTCTTTATACTACCAAGG-3′) and a PGK-promoter–specific primer (5′-TGCTAAAGCG-CATGCTCCAGACTG-3′). Frequency of the homologous recombinations was 25.7% with pbKO-NEO at first-round transfection and 20.0% with pbKO-HYG at second-round transfection.

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18. Assay was performed in 96-well, V-bottom plates (Costar, Cambridge, MA) with 200 μl of RPMI-1640 that contained 10% fetal calf serum (in triplicate). After the cells were harvested, they were counted and stained for flow cytofluorometric analysis.
19. We thank P. Mombaerts, E. George, and R. Hynes for ES cell lines, H. Rayburn for technical advice, A. Berns for the PGK-hyg-poly(A) cassette, and H. Nakauchi for helpful discussions. Supported by the Howard Hughes Medical Institute (D.Y.L. and F.W.A.), NIH grant AI 15322 (D.Y.L.), Uehara Foundation (I.N. and K.K.), and the Leukemia Society of America (P.J.L.).

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Immunoglobulin V_H3 Gene Products: Natural Ligands for HIV gp120

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Infection with human immunodeficiency virus–type 1 (HIV-1) depletes T cells expressing CD4 and B cells expressing immunoglobulin (Ig) V_H3 gene products. A subpopulation of normal B cells from non–HIV-infected individuals was shown to bind to HIV gp120 by means of membrane Ig; most of these B cells expressed V_H3 family Ig. Serum V_H3 IgM from uninfected individuals also avidly bound gp120. Finally, gp120 selectively induced Ig secretion by V_H3 B cells, indicating that the binding of gp120 functionally activated these cells. These results indicate that naturally occurring V_H3 Ig is a second ligand for gp120 and a candidate superantigen for V_H3 B cells.

Acquired immunodeficiency syndrome (AIDS) is characterized by dysfunction and depletion of immune cells including CD4$^+$ T cells and B cells (*1*, *2*). CD4$^+$ T cells are selectively deleted as a result of a series of events initiated by the binding of HIV to the CD4 molecule by means of the viral envelope glycoprotein gp120 (*3*). The exact mechanism by which binding leads to deletion of this population is unknown. However, several models have been proposed, including direct cytotoxicity (*4*), gp120-induced polyclonal apoptosis (*5*), and oligoclonal superantigen-mediated deletion (*6*).

We reported a clonal deficit of B cells bearing rearranged Ig V_H3 genes in individuals with AIDS (*7*). V_H3 is the largest family of Ig heavy chain variable regions consisting of 30 or more related gene segments (*8*). Analogous to the situation with CD4$^+$ T cells, a model for V_H3 B cell depletion could involve direct binding of HIV to this B cell population. We therefore examined whether HIV gp120 bound to a subpopulation of normal B cells. Mononuclear cells were obtained from fresh tonsil tissue from subjects aged 2 to 14 years as described (*9*). All of these patients were seronegative for HIV. Tonsil cells were incubated with recombinant gp120 from HIV-1_{SF2} [expressed in Chinese hamster ovary (CHO) cells], stained with antisera to HIV-1_{SF2} gp120 and lineage-specific markers, and analyzed by flow cytometry (*10*). In all eight subjects, 3 to 6% of the B cell population (CD19$^+$) bound gp120 (Fig. 1A). T cells in the tonsil served as an internal control for gp120 binding. As expected, 68% of the T cell (CD19$^-$, CD3$^+$) population bound gp120, consistent with the known abundance of CD4$^+$ T cells (Fig. 1A). In some experiments, tonsils were initially depleted of T cells by erythrocyte rosetting (*9*). This procedure did not diminish the population of CD19$^+$ cells binding gp120.

Although normal B cells do not express CD4, it was conceivable that this small population of B cells might express this molecule. However, we were unable to detect CD4 on the surface of CD19$^+$ tonsil cells (*11*). Furthermore, pretreatment of tonsil cells with Leu3a, an antibody to CD4 (anti-CD4) that blocks the binding of gp120 to CD4 (*12*), completely inhibited gp120 binding by tonsil T cells (Fig. 1B). In contrast, Leu3a did not inhibit binding of gp120 to the subpopulation of CD19$^+$ cells (Fig. 1B), demonstrating that gp120 did not bind to the B cells by means of CD4. Consistent with these results, we observed that a similar B cell subpopulation also bound nonglycosylated HIV-1_{SF2} gp120 produced in yeast (*11*), a form of gp120 that does not bind to CD4 (*13*).

We postulated that gp120 bound to these normal B cells by means of membrane Ig. To address this idea, we selectively removed cell surface Ig by pretreating tonsil cells with antibodies to κ and λ Ig light chain (anti-κ and anti-λ) before the gp120 binding assay. Endocytosis of membrane Ig with antibody to Ig decreases surface Ig by >90% (*14*); in the present experiments, such treatment removed 94% of membrane IgD (Fig. 1C). After removal of membrane Ig, a corresponding amount of gp120 binding was lost (Fig. 1D), indicating that gp120 binding was associated with membrane Ig.

To determine whether the B cells that

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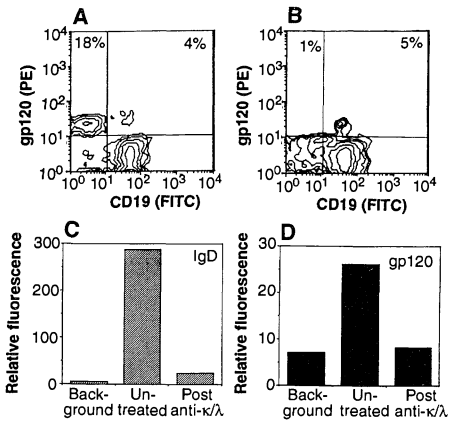

Fig. 1. (**A**) Detection of a minor population of CD19$^+$ gp120-binding cells. Mononuclear human tonsil cells were sequentially stained with HIV-1_{SF2} gp120 (*10*), goat antibody to gp120, swine anti-goat IgG-PE, and anti-CD19–FITC. Bound to gp120 were 4% of B cells (CD19$^+$) and 68% of T cells (CD19$^-$). Staining with antibody to CD20 and antibody to CD3 confirmed the identity of these cell types (CD20$^+$/CD3$^-$ and CD20$^-$/CD3$^+$, respectively) (*31*). Representative of eight subjects. (**B**) Effect of anti-CD4 pretreatment on gp120 binding. Tonsil cells were pretreated with Leu3a (32 ng, 30 min at 4°C), washed, then stained as in (A). T cell binding to gp120 (upper left quadrant); B cell binding to gp120 (upper right quadrant). Representative of three experiments. (**C** and **D**) Effect of membrane Ig endocytosis on gp120 binding. Tonsil cells were pretreated with anti-human κ and λ light chain to elicit membrane Ig endocytosis then washed and stained for CD19 and either membrane IgD (C) or gp120 binding (D). Values are the mean fluorescence calculated from flow cytometric data on gated CD19$^+$ cells. Background staining was determined with fluorochrome-labeled isotype control antibodies. Representative of three experiments.

Table 1. Predominance of V_H3 idiotopes in gp120-binding B cells. Tonsil B cells from normal subjects were stained for CD19, gp120, and V_H3, V_H1, or $V_κIIIa$ idiotopes then analyzed by flow cytometry, gating on either all CD19$^+$ cells (all B cells) or CD19$^+$ gp120-binding cells (gp120$^+$ B cells) (*10*). An example of one representative V region recognized by the anti-idiotope is shown in parentheses. Results of one experiment are shown; similar results were obtained in three separate experiments.

V family (representative gene)	Anti-idiotope	Percent of all B cells	Percent of gp120$^+$ B cells
V_H3 (*V_H26*)	16/6	16	26
V_H3 (*1.9III*)	B6 and D12	12	46
V_H3 (*9-1*)	BK2	3	5
V_H1 (*humvh1051*)	G6	2.5	<0.1
$V_κIIIa$ (*humvk328*)	IV.6	8	<0.1

bound gp120 expressed V_H3 Ig, we utilized four antibodies to the V_H3 idiotype (anti-idiotype), B6, D12, BK2, and 16/6 (*10, 15*). Together, these antibodies recognize about 11 germline-encoded V_H3 gene products (*8*). An overabundance of V_H3 idiotope–positive B cells was detected in gp120-binding B cells, and these four idiotopes alone accounted for 77% of the gp120-binding B cell population (Table 1). In contrast, this population did not include cells positive for G6 (Table 1), an antibody to an idiotope for a subset of V_H1 gene products (*15*).

For confirmation of the identity of this population of B cells, we examined the binding of gp120 by tonsil mantle zone B cells immortalized with the Epstein-Barr virus (EBV) (*16*). The EBV-immortalized B cells were stained with gp120 (1.5% gp120$^+$ cells) and sorted by a fluorescence-activated cell sorter (FACS) into gp120-binding and gp120-nonbinding populations. The gp120-binding phenotype of these sorted populations remained stable after expansion for 3 weeks in culture: the gp120-nonbinding population still did not bind gp120, whereas 71% of the gp120-binding population remained gp120$^+$ (18% brightly, 53% more dimly) (Fig. 2). EBV-immortalized tonsil germinal-center cells lacked detectable binding to gp120 (*11*), consistent with the normal deficit of V_H3 B cells at this location (*9*).

The preceding experiments suggested that an Ig prevalent in normal individuals was capable of binding to gp120. To directly test this idea in a cell-free system, we evaluated binding of soluble Ig to gp120 with an enzyme-linked immunosorbent assay (ELISA) (*17*). Serum was collected from normal, seronegative individuals with no history of HIV contact (*18*). We observed a dose-dependent binding of serum IgM to gp120 (Fig. 3A). There was significant binding to gp120 with as little as 5 to 10 ng of serum IgM. Binding by IgM was about 10 times the binding by IgG (Fig. 3A). We obtained similar results when we used a commercially available ELISA plate to detect antibodies against a total HIV-1 lysate (Retro-tek HIV-1 ELISA kit). With this ELISA plate, binding was readily detectable for normal serum IgM, but not for IgG; IgG is the isotype routinely assayed by this kit (*11*).

We analyzed gp120 binding by IgM fractions that had been enriched or depleted of V_H3 Ig by means of *Staphylococcus aureus* protein A. In addition to its well-known binding domain for IgG constant regions, protein A contains a second distinct domain that binds to a conserved V_H peptide present in most V_H3 Ig (*19*). Normal serum was first depleted of IgG with protein G beads then fractionated with a protein A column (*20*). The gp120-binding activity of IgM was enriched in the protein A–bound fraction and depleted in the unbound fraction (Fig. 3A). It was significant that the IgM from the protein A–bound fraction avidly bound gp120 from different HIV strains and different recombinant expression systems (*21*). This pattern distinguishes them from the strain-specific antibodies present in HIV seropositive individuals. In addition, binding of both glycosylated and nonglycosylated gp120 indicated that the recognized epitope was peptide encoded.

To examine the binding of a monoclonal IgM to gp120, we utilized a set of monoclonal IgM purified from sera of patients with Waldenström's macroglobulinemia, a clonal malignancy of IgM-secreting B cells (*22*). Monoclonal IgM from patient HEA (V_H3, $V_κ3$) bound gp120 with a

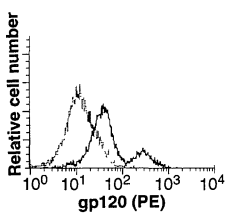

Fig. 2. The binding of gp120 by FACS-sorted EBV-immortalized B cells. EBV-immortalized mantle zone tonsil cells were FACS-sorted into gp120-binding and gp120-nonbinding populations and then reanalyzed after expansion in culture for 3 weeks (*16*). The fluorescence histogram for sorted gp120$^+$ B cells is shown by the solid line. The gp120$^-$ B cells or background staining (all reagents except gp120) were dim and superimposable; both are shown by the stippled line.

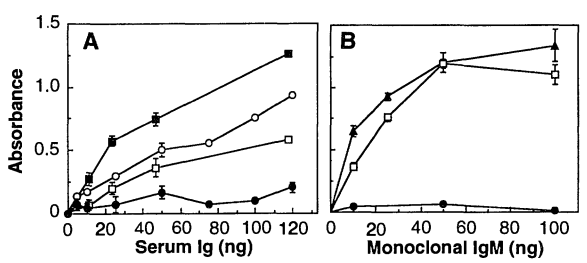

Fig. 3. Binding of soluble Ig to gp120. Binding of Ig to HIV-1_{SF2} rgp120 was measured by ELISA (*17*). (**A**) Normal serum assayed with antibody to IgM (open circles) or antibody to IgG (closed circles). Representative of sera from eight individuals. IgG-depleted normal serum Ig was separated into fractions enriched (closed boxes) or depleted (open boxes) for protein A binding (*20*). Representative of three fractionation experiments. (**B**) Monoclonal IgM binding to gp120. HEA (square); SMI (circle); and normal serum (triangle). In both figures values are the mean of triplicate determinations, and error bars indicate standard deviations.

similar dose-dependence as serum IgM (Fig. 3B); this IgM also bound to protein A (*11*). The binding affinity of HEA to glycosylated HIV_{SF2} gp120 was determined by the method of Friguet *et al.* (*23*) yielding a dissociation constant (K_d) of 8.6×10^{-9} M. In contrast, the monoclonal IgM SMI (V_H1, $V_\kappa3$) did not bind gp120 (Fig. 3B) or protein A (*11*).

Because V_H3 Ig binds gp120, we predicted that gp120 should successfully substitute as a conventional membrane Ig ligand for the V_H3-expressing B cell population. We cultured tonsil B cells in an established system that is responsive to direct membrane Ig stimulation (*24*) to test the functional consequences of gp120 binding. Incubation of the tonsil B cells with gp120 induced a striking increase in Ig secretion by $D12^+$ V_H3 B cells, comparable in magnitude to the stimulation induced by antibody to IgD (anti-IgD) (Fig. 4). In contrast, whereas $IV.6^+$ V_κIIIa B cells were responsive to anti-IgD, gp120 failed to induce Ig secretion over basal amounts. This unresponsiveness was consistent with the paucity of gp120-binding B cells in this clonal subpopulation and was also notable because the abundance of IV.6 and D12 B cells in tonsil is similar (Table 1).

In this study, we have identified a second cellular ligand for gp120. The binding of gp120 to naturally occurring Ig has several features resembling a superantigen interaction. T cell superantigens characteristically bind to the β chain of the T cell receptor (TCR) and to major histocompatibility complex class II proteins outside the antigen-binding groove (*25*). Binding of a superantigen is also correlated with an initial stimulation and subsequent depletion of a relatively large population of T cells that express specific TCR β chains (*25*). The idea of superantigens for B cells has been proposed from the observed V_H-specific binding of *S. aureus* bacterial toxin protein A (*19*) and certain autoantigens (*26*) and is consistent with the conserved structural motifs of framework regions in Ig families and clans (*27*). Analogous to these precedents, gp120 binds to a subpopulation of B cells sharing a restricted Ig V gene family (V_H3) irrespective of light chain isotype (*11*). Although the V_H3 genes detected in this population (homologs of *1.9III*, V_H26, and *9-1*) are highly diverse in their complementary determining regions, they share conserved, surface-exposed peptide segments in FR1 and FR3. This observation provides further evidence for the hypothesized role of these sites as an alternate antigen-binding site on the Ig molecule (*27*) possibly with multireactive features (*28*). Functionally, binding of gp120 activates V_H3 B cells in vitro. In AIDS patients, there is a clonal deficit of V_H3-expressing B cells; interestingly, this clonal deficit is preceded by an expansion of the V_H3 B cell pool in earlier clinical stages of HIV infection (*11*), indicating that the seemingly polyclonal B cell stimulation that accompanies HIV infection (*29*) preferentially involves the V_H3 B cell population.

These findings may be relevant to HIV pathogenesis. The immunologic sequelae of receptor-mediated binding of HIV or soluble gp120 to the relatively large V_H3 B cell population may contribute to HIV-associated B cell derangements. Furthermore, initial studies indicate that V_H3-enriched IgM from non–HIV-infected individuals is neutralizing for HIV infection of T cells in a serum-free in vitro assay (*11*); in vivo, the concentrations of anti-gp120 IgM and V_H3 IgM correlate with the clinical stage of HIV infection (*11*). These findings suggest a role for serum V_H3 IgM in the course of HIV infection and, consequently, the potential importance of considering V_H3 B cells in strategies for HIV vaccine development.

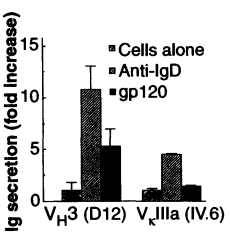

Fig. 4. Ig secretion by $D12^+$ B cells stimulated with gp120. Purified tonsil B cells were cultured on mitomycin-treated CDw32 L cells for 6 days in a T cell–independent system supplemented with recombinant human interleukin-4 (3 ng/ml) and mAb to CD40 G28-5 (50 ng/ml) as described (*24*). To some samples 100 ng of $HIV\text{-}1_{SF2}$ gp120 or anti-IgD (0.5 μg/ml) were added. We determined Ig secretion by ELISA. Results are displayed as the fold increase above basal amounts (8.4 ± 6.6 ng for D12 and 1.1 ± 0.3 ng for IV.6) of Ig secretion. On gp120 stimulation, the D12 detected 52% of the total Ig secretion (basal amount of total Ig = 34.0 ± 8.0 ng).

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10. Tonsil mononuclear cells were isolated (*9*) and resuspended in Hanks salts with 2% heat-inactivated fetal calf serum, 10 mM Hepes, and 0.02% sodium azide. Cell viability was assessed by trypan blue exclusion, and only cell populations that were >95% viable were used. Cells were prepared for flow cytometry analysis (*9*) with the following modifications: 5×10^5 cells were incubated with recombinant gp120 (4 μg/ml) from $HIV\text{-}1_{SF2}$ (expressed in CHO cells) (AIDS Research and Reference Reagent Program, Division of AIDS, National Institute of Allergy and Infectious Diseases, National Institutes of Health, [ARRRPDANN]: catalog number 386) (*30*) for 30 min at 4°C. In one experiment (*11*) nonglycosylated gp120 was used (from $HIV\text{-}1_{SF2}$, expressed in yeast; ARRRPDANN catalog number 388) [N. L. Haigwood, *AIDS Res. Hum. Retroviruses* **6**, 855 (1990)]. Cells were incubated for 30 min (4°C) with antiserum to $HIV\text{-}1_{SF2}$ gp120 (ARRRPDANN catalog number 385) (*30*) and subsequently with one or more of the following: swine antibody to goat IgG conjugated to phycoerythrin (PE) (anti-goat IgG–PE) (Caltag), or fluorescein isothiocyanate (FITC)–conjugated antibodies to CD19 (anti-CD19–FITC), CD20, or CD3 (Becton Dickinson). In some experiments, cells were incubated with the murine monoclonal antibodies B6, D12, G6, 16/6, and IV.6 [see (*15*)] to V_H3 or $V_\kappa III_a$ idiotopes. The mouse monoclonal IgG BK2 was produced in our laboratory against the *9-1* gene product (clone LJ86) [J. Braun *et al.*, *J. Clin. Invest.* **89**, 1395 (1992)]. The specificity of BK2 was verified with 15 sequenced human monoclonal antibodies. Cells were analyzed with a FACScan flow cytometer. We excluded dead cells on the basis of forward and side scatter and with the dead cell discriminator 7-aminoactinomycin D [I. Schmid, W. K. Krall, C. H. Uittenbogaart, J. Braun, J. V. Giorgi, *Cytometry* **13**, 204 (1992)]. Control samples were processed in a similar fashion with the omission of gp120 or substitution of a FITC-conjugated isotype control antibody, or both. Results show the fluorescence intensity of 5000 live cell events.
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16. T cell–depleted mononuclear tonsil cells were sorted into a germinal center ($Leu\text{-}17^{bright}$ and IgD^{dim}) and mantle zone (IgD^{bright} and $Leu\text{-}17^{dim}$) subpopulations with a $FACSTAR^{plus}$ cell sorter (Becton Dickinson) and immortalized with EBV. After expansion in culture for 3 weeks, the EBV-immortalized mantle zone B cells were stained with gp120 (*10*) and FACS-sorted into subpopulations that bound and that did not bind gp120. The sorted cells were again expanded in culture for 3 weeks and then reanalyzed for gp120 binding.
17. ELISA microtiter plates (Costar) were coated overnight (4°C) with 50 ng of gp120 diluted in carbonate-bicarbonate buffer, pH 9.6 (Sigma). The plates were rinsed three times for 15 min with phosphate-buffered saline (PBS) plus 0.5% Tween-20; incubated for 1 hour (25°C) with normal serum, protein A–enriched or –depleted fractions, or monoclonal IgM diluted in PBS and 0.5% Tween-20; and stained for 1 hour (4°C) with goat antibody to human IgM (anti-human IgM)– or IgG–horseradish peroxidase (HRP) (Southern Biotechnology Associates). Qualitatively similar results were obtained if 3% nonfat milk or bovine serum albumin (1 to 5 mg/ml) was used instead of Tween-20. Samples were incubated with 50 μl of *o*-phenylenediamine (OPD) dihydrochloride (Sigma) for 30 min (37°C). We then added 50 μl of 3 N sulfuric acid and determined the absorbance at 492 nm. The following controls had no binding or

color reaction: (i) the absence of serum, protein A fractions, or monoclonal IgM, (ii) substitution of anti-human IgM–HRP with HRP-conjugated antibody to mouse IgM, and (iii) the absence of gp120 (secondary and tertiary antibodies did not bind to the ELISA plate in the absence of carbonate-bicarbonate buffer). The following additional experiments ruled out artifactual binding due to ELISA conditions: (i) an immuno-dot blot assay in which the blotting of gp120 onto nitrocellulose and the subsequent Ig-binding reaction were each done at neutral pH, and (ii) the HEA-gp120 K_d determination [see (*23*)] demonstrated liquid-phase binding of antibody and antigen. In addition, because this gp120 preparation was a highly pure, native conformation product (Chiron lot MGC022; >90% by SDS–polyacrylamide gel electrophoresis gel scan, 100% binding to soluble CD4), the high-avidity competition indicates that this V_H3 IgM binds to gp120 in its native conformation. In this context, binding to nonglycosylated gp120 suggests that V_H3 IgM recognizes epitopes present on both native and denatured gp120.

18. Normal serum samples from HIV seronegative individuals were obtained from the University of California, Los Angeles (UCLA) blood donor facility. Six different serum samples tested displayed similar gp120-binding activity in our assay conditions. Monoclonal IgM samples from patients with Waldenstrom's macroglobulinemia were negative for IgG-specific HIV reactivity as tested by the UCLA Center for Health Sciences Clinical Laboratory.
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21. Recombinant gp120 proteins from the following HIV-1 or HIV-2 strains were tested for binding to Ig: SF2, SF2 (nonglycosylated), JRFL, JR-CSF, ST, Ba-L, and BH10 (*10*) (D. W. Brighty, M. Rosenberg, I. S. Y. Chen, M. Ivey-Hoyle, *Proc. Natl. Acad. Sci. U.S.A.* **88**, 7802 (1991); M. Ivey-Hoyle *et al.*, *ibid.* p., 512.
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IRS-1: Essential for Insulin- and IL-4–Stimulated Mitogenesis in Hematopoietic Cells

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Although several interleukin-3 (IL-3)–dependent cell lines proliferate in response to IL-4 or insulin, the 32D line does not. Insulin and IL-4 sensitivity was restored to 32D cells by expression of IRS-1, the principal substrate of the insulin receptor. Although 32D cells possessed receptors for both factors, they lacked the IRS-1–related protein, 4PS, which becomes phosphorylated by tyrosine in insulin- or IL-4–responsive lines after stimulation. These results indicate that factors that bind unrelated receptors can use similar mitogenic signaling pathways in hematopoietic cells and that 4PS and IRS-1 are functionally similar proteins that are essential for insulin- and IL-4–induced proliferation.

Insulin receptor substrate–1 (IRS-1) is a hydrophilic protein that undergoes tyrosine phosphorylation immediately after insulin stimulation (*1*). It contains 20 phosphorylation sites for tyrosine and 30 for threonine and serine but possesses no apparent identity with other known proteins (*2, 3*). Tyrosine-phosphorylated sites within IRS-1 associate with high affinity to cellular proteins that contain Src homology 2 (SH2) domains, including phosphatidylinositol (PI)-3 kinase, growth factor receptor–bound protein 2 (GRB2) (Sem-5), and SH2-containing protein tyrosine phosphatase-2 (SH-PTP2) (*2, 4–6*). PI-3 kinase is activated when the SH2 domains in its 85-kD regulatory subunit (p85α) bind to tyrosine-phosphorylated IRS-1 (*7, 8*). Overexpression of IRS-1 in CHO cells enhances insulin- and insulin-like growth factor (IGF-1)–stimulated mitogenesis, and microinjection of recombinant IRS-1 into *Xenopus* oocytes mediates insulin-stimulated oocyte maturation (*9*). Insulin receptor mutants that are defective for mitogenic signaling are also incapable of inducing IRS-1 phosphorylation (*2, 10*). Although these studies imply that IRS-1 is a component of the insulin signaling pathway, they do not provide direct evidence that expression of this protein is required for insulin action.

Although the IL-3–dependent myeloid progenitor cell lines FDC-P1 and FDC-P2 do not express IRS-1, in these cells IL-4, insulin, and IGF-1 stimulate potent tyrosine phosphorylation of a protein, designated 4PS (IL-4–induced phosphotyrosine substrate) (*11, 12*). This substrate behaves like IRS-1 in that it strongly associates with p85α of PI-3 kinase after factor stimulation and migrates at 165 to 175 kD during SDS–polyacrylamide gel electrophoresis (*11, 12*). The 4PS protein is weakly recognized by a polyclonal antibody directed against IRS-1, and the phosphopeptide patterns generated by V8 digestion of the two proteins are similar but not identical (*11*). The concentrations of insulin and IL-4 required to induce mitogenesis in the FDC lines correlate with those needed to stimulate tyrosine phosphorylation of 4PS, which suggests that this IRS-1–like substrate might participate in IL-4– and insulin-mediated signal transduction in hematopoietic cells.

The 32D line has a myeloid progenitor phenotype and is IL-3–dependent like the FDC lines (*13*), but 32D cells did not proliferate upon exposure to IL-4 or insulin. Induction of DNA synthesis by IL-4 in 32D cells was negligible, and insulin had no detectable mitogenic effect at any concentration tested (Fig. 1). In contrast, IL-4 and insulin induced efficient DNA synthesis in the FDC-P2 line (Fig. 1). Receptor expression was examined by saturation binding assay: 32D cells had ~1200 IL-4 and ~500 insulin receptors per cell and FDC-P2 cells had ~1800 IL-4 and ~750 insulin receptors per cell (*14*). Because IL-4 and insulin receptor levels in 32D cells were only slightly lower than those in FDC-P2 cells, it seemed unlikely that small receptor num-

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