Human B Cells Express Two Types of Interleukin-1 Receptors

By David Benjamin and Steven K. Dower

Several reports demonstrate that interleukin-1α (IL-1α) and interleukin-1β (IL-1β), although distantly related at the primary sequence level, bind to the same receptor. This raises the question of what separation, if any, there might be between their biologic activities. Receptor binding assays and cross-competition studies in three Epstein Barr virus (EBV) positive cell lines showed that a subset of IL-1 receptors may bind IL-1α preferentially and that a separate subset may bind IL-1β preferentially. Crosslinking studies with 125I-IL-1α or 125I-IL-1β demonstrated the expression of both relative molecular mass (M,) 80,600 IL-1 receptor, known to be T cell-specific, and M, 69,500 IL-1 receptor, recently detected on B-cell lineage only. Whether the high affinity binding sites correspond to M, 80,500 and the low affinity binding sites to M, 69,500 remains to be elucidated. © 1990 by The American Society of Hematology.

THE PURIFICATION, cloning, and expression of cDNAs for murine and human interleukin-1α (IL-1α) and interleukin-1β (IL-1β) have made possible the direct analysis of the chemistry, structure-function relationships, and general biology of this family of immunoregulatory proteins (reviewed in reference 1). As information accumulated from studies using purified natural and recombinant IL-1 (rIL-1), it became clear that IL-1α and IL-1β each mediate most, if not all, of the wide range of activities previously ascribed to IL-1.1,2 The cause of this identical spectrum of biologic activities is the existence of a single class of plasma membrane IL-1 receptors that bind both IL-1α and IL-1β on all IL-1-responsive cell types tested so far, including murine T cell lines, murine thymocytes, human peripheral blood T lymphocytes, and human diploid fibroblasts.3,4 Structural characterization by ligand crosslinking has demonstrated that the membrane IL-1 receptor on murine T cells, murine fibroblasts, bovine aortic endothelial cells, human diploid fibroblasts, and rat hepatocytes has a relative molecular mass (M,) of 82,000.5 In contrast, IL-1 receptors on the Epstein Barr virus (EBV)-transformed human B cell lines VDS-O and 3B6, the EBV-positive Burkitt’s lymphoma cell line Raji, and the pre-B cell line 70Z/3, displayed M, of 60,000 to 68,000.9,14 These data suggest that IL-1 receptors expressed on B cells are different from those detected on other cell types. Moreover, comparison of the biochemical properties and kinetic analysis of the IL-1 receptor in the Raji B cell line to EL-4 murine T lymphoma cell line showed that Raji cells had lower binding affinity but much higher receptor density per cell than a subclone of EL-4 T cells.12 Raji cells also failed to internalize IL-1 and demonstrated altered receptor binding affinities with IL-1 analogs.12 These studies show that not all cells share a common IL-1 receptor.

The goal of the present study was to examine IL-1 receptors on human B cell lines in an attempt to further elucidate the differences between IL-1 receptors on B cells compared with T cells and other cell types. Our data indicate that human B cells can express distinct IL-1 receptors. Whether these distinct affinity binding sites are shared by a single molecule or by various chains remains to be elucidated. Further, we suggest that, under some circumstances, B cells can express the IL-1 receptor subtype previously supposed to be T cell lineage-specific.

MATERIALS AND METHODS

Cell lines. The study included tumor cell lines derived from a patient with AIDS and Burkitt’s lymphoma (PA682BM-2) and EBV-transformed cord blood (CB) lymphocyte cell lines (CB23 and CB33). Methods of derivation and documentation of the origin of these B cell lines have been previously reported.15 The cell lines were maintained as suspension culture in RPMI 1640 plus 10% fetal calf serum (Whittaker Bioproducts, Walkersville, MD) at 37°C in 5% CO2 and were subcultured every 3 to 4 days. These B cell lines were screened by a panel of B- and T-cell surface markers (HLA-DR, Leu-10, BA-1, B-1, B-2, J-5, OKT10, OKT3, OKT11, and surface immunoglobulin), and were positive for B lymphocyte markers only.

Radiolabeling of rIL-1α and rIL-1β. Recombinant human IL-1α and IL-1β were labeled as previously described.16 Briefly, rIL-1β was labeled with di-iodo (125I) Bolton-Hunter reagent (New England Nuclear, Glenolden, PA). Ten micrograms (0.57 nmol) of protein in 10 µL of phosphate (0.015 mol/L)-buffered saline (PBS; 0.15 mol/L), pH 7.2, was mixed with 10 µL of sodium borate (0.1 mol/L)-buffered saline (0.15 mol/L), pH 8.5, and reacted with 1 mCi (0.23 nmol) of Bolton-Hunter reagent according to the manufacturer’s instructions for 12 hours at 8°C. Subsequently, 30 µL of 2% gelatin and 5 µL of 1 mol/L glycine ethyl ester were added, and the protein was separated from unreacted Bolton-Hunter reagent on a 1 mL bed volume Biogel P6 column (Bio-Rad Laboratories, Richmond, CA). Routinely, 50% to 60% incorporation of label was observed. Recombinant IL-1α was labeled with Na+ (125I) iodide using a modified chloramine-T method. Briefly, 10 µg of rIL-1α (0.57 nmol) in 10 µL of phosphate-buffered saline was added to 2.5 mCi (1.0 nmol) of sodium (125I) iodide in 25 µL of 0.05 mol/L sodium phosphate, pH 7.0. Reaction was initiated by addition of 30 µL of 1.4 × 10−4 mol/L chloramine-T (4.2 nmol, Sigma, St Louis, MO). After 30 minutes on ice, the reaction mixture was fractionated by gel filtration as described above. Routinely, 40% to 50% of 125I was incorporated into protein. Precipitation of the 125I–IL-1α preparations, radiiodination yielded specific activities in the range of 1 × 1010 to 5 × 1013 cpm/mmol (0.4 to 2 atoms I per molecule protein), and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) revealed a single band.306, Oklahoma City, OK 73190.

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labeled polypeptide of 17.5 Kd, consistent with previously reported values for IL-1.

The fact that both labeled proteins were greater than 98% TCA precipitable indicates that the $^{125}$I was covalently bound to protein. Comparison of their biologic activities on murine T lymphoma cells demonstrated that recombinant IL-1β suffered some loss of activity on labeling, but IL-1α retained 100% biologic activity after labeling, and greater than 95% could be bound by IL-1 receptor-bearing cells.

**Binding assay of $^{125}$I-labeled IL-1 to cells.** Human B cells, maintained as described above, were harvested and washed twice with cold (4°C) "binding" medium, which consisted of RPMI 1640 medium containing 5% fetal calf serum and 25 mmol/L HEPES (Sigma). Washings were done to remove IL-1α secreted by these cell lines and thus eliminate its autobinding to cell surface IL-1 receptors. Then cells and $^{125}$I-IL-1 were incubated in binding medium at a concentration of $9 \times 10^6$ cells/mL and the appropriate concentration of $^{125}$I-IL-1 in a total volume of 300 µL, at 4°C. Incubation was carried out on a rocker platform to ensure continuous mixing of cells and radioactive ligand. Nonspecific binding of $^{125}$I-IL-1 was measured by incubation in the presence of at least 100-fold excess of unlabeled rIL-1, at a dose of unlabeled ligand predetermined to lead to 100% receptor occupancy. At the end of the incubation, bound and free $^{125}$I-IL-1 were separated by removing triplicate 100 µL aliquots from the incubation mixture, layering these on 300 µL of phthalate oil mixture in 400 µL polyethylene centrifuge tubes (American Scientific Products, Dallas, TX), and centrifuging for 1 minute at 12,000g in a Beckman microfuge B. The tube tip containing the cell pellet was excised, and cell-associated radioactive activity was determined in a gamma counter. The results were expressed as the mean cpm (specific binding); data were subsequently converted to molecules per cell (bound) or molar concentrations (free) for analysis.

**Competitive binding experiments.** To test directly whether rIL-1α and rIL-1β bind to the same receptor, we conducted inhibition experiments in which the binding of each radiolabeled IL-1 preparation to the cells was inhibited by unlabeled IL-1α from the same stock solution and from the other two unlabeled IL-1 preparations. Cells were incubated with a fixed concentration of each type of $^{125}$I-IL-1 and various concentrations of unlabeled rIL-1α or rIL-1β, respectively, in 300 µL of binding medium, for 2 hours at 4°C on a rocker platform. Specific activities of $^{125}$I-IL-1 used were: with PA682BM-2 cells: rIL-1β, 4.6 x 10^5 cpm/mmol-1, and rIL-1α, 1.5 x 10^5 cpm/mmol-1; with CB23 cells: rIL-1β, 8 x 10^4 cpm/mmol-1, and rIL-1α, 3 x 10^5 cpm/mmol-1; with C33 cells: rIL-1β, 4.6 x 10^5 cpm/mmol-1, and rIL-1α, 1.5 x 10^5 cpm/mmol-1. At the end of the assay, cells and bound radiolabel were separated from unbound label as described for direct binding experiments.

**Data analysis.** Binding data were analyzed with models for noncooperative binding to one or two types of cell surface receptors, as described elsewhere. Inhibition data were analyzed with an equation describing competition of two types of ligand for one class of cell surface sites, as described elsewhere. All data analysis was performed by nonlinear least squares fitting of models to data in real space; that is, using the data in the form bound (molecules per cell) vs free (molar), or percent inhibition (1%) versus competitor concentration. Theoretical curves were calculated from the best fit parameter values.

All data analysis and curve plotting were done using RS/1, an interactive graphics and data management package (IBM Software Products Corp, MA) running on a VAX 11/750 under the VMS Operating System.

**Crosslinking studies.** $^{125}$I-IL-1α and $^{125}$I-IL-1β were crosslinked as previously described. Briefly, 3.2 x 10^6 cells were incubated in binding medium for 1 hour at 4°C with 1.5 x 10^-9 mol/L of $^{125}$I-IL-1α or $^{125}$I-IL-1β in the presence or absence of 1 x 10^-7 mol/L unlabeled IL-1α or IL-1β, respectively. At the end of the incubation, the cells were centrifuged (1,000 rpm for 10 minutes) and resuspended in 1 mL of PBS (pH 7.2), and 20 µL of 50 µg/mL disuccinimidyl suberate (Pierce Chemical Co, Rockville, IL) in DMSO was added to a final concentration of 1 mg/mL. The samples were incubated for 15 minutes at room temperature (~22°C), subsequently washed once with binding medium, twice with PBS, and finally resuspended in 50 µL of PBS containing 1% Triton X-100 and 2 mmol/L phenylmethylsulphonyl fluoride (PMSF) or a mixture of protease inhibitors (pepsatin, 2 mmol/L final [Sigma], O-phenanthroline, 2 mmol/L final, and PMSF, 2 mmol/L final). Protease inhibitors were used to prevent possible proteolytic degradation of the $^{125}$I-IL-1/IL-1 receptor complex, which may occur upon its extraction from the cell. The detergent extraction was performed for 15 minutes on ice, and nuclei and other debris were subsequently removed by centrifugation for 10 minutes in a microfuge. Supernatant (40 µL) was removed and analyzed by electrophoresis on 8% polyacrylamide slab gels, as described previously. The IL-1α and IL-1α/IL-1β receptor complexes were subsequently detected by contact autoradiography of the dried gel.

**RESULTS**

**Radiolabeling and cross-competition studies.** Figures 1, 2, and 3 show binding of both IL-1α and IL-1β to the cell lines CB23, CB33, and PA682BM-2, respectively, and Table 1 summarizes the analyses of the data presented in these figures. On all three cell lines, IL-1α detected a similar number of sites with an affinity in the range of 5 x 10^8 mol^-1. This affinity is similar to that reported for a variety of other cell lineages. In addition, a second class of receptors of approximately 50-fold lower affinity were present on CB23, in considerably larger numbers (1,600 sites per cell), and some low affinity binding was also detectable on the other two cell lines. The data for PA682BM-2 and CB33, however, were insufficient to permit the separate determination of R and K for the low affinity receptors on these cells. If it is assumed that on these cells also the K_a was 1 x 10^8 mol^-1 (as for CB23), then the low affinity site numbers for IL-1α on these lines would be approximately 100 for the PA682BM-2 line, and approximately 1,000 for CB33. These arguments would suggest that the total number of IL-1α binding sites...
Scatchard plot analysis defined in Fig 1.

The case of CB33 does there appear to be some low affinity present on CB23 with a $K_a$ close to $10^9$ mol$^{-1}$. This is consistent with previous values reported for human B cells. Similar observations were made for PA682BM-2. Only in the case of CB33 does there appear to be some low affinity binding, for which $K_a$ and $R$ cannot be resolved (Table 1), in addition to there being approximately 1,100 sites with $K_a$ of approximately $10^9$ mol$^{-1}$. It is clear from both the IL-1α and IL-1β data sets that interaction of IL-1 with receptors on these human B cell lines can give rise to complex binding isotherms. Such patterns have been previously reported by others for murine T cells. The functional and structural significance of this heterogeneity is at present unclear, particularly since the IL-1 receptor identified by cDNA cloning appears to be composed of a single glycosylated polypeptide chain.

While the heterogeneity of both IL-1α and IL-1β binding raises the possibility that there might be distinct and/or crossreactive subsets of receptors for the two interleukins, the direct binding data do not clearly resolve this issue. In particular, inspection of the parameter values in Table 1 shows that the total number of IL-1α binding sites for CB23 (1,600 to 2,100), PA682BM-2 ($\sim 400$), and CB33 ($\sim 1,300$) are similar to those sites binding IL-1β with $K_a$ $\sim 10^6$ mol$^{-1}$, in each instance: 2,400 for CB23, $\sim 320$ for PA682BM-2, and $\sim 1,100$ for CB33. The most reasonable interpretation of these data would be that in each case the receptors bind both IL-1α and IL-1β. This hypothesis is broadly confirmed by a series of cross-competition experiments (Figs 4 through 6).

The inhibition constants ($K_i$) and maximal levels of blockade for each cell line, $^{125}$I-IL-1α/IL-1β combination are summarized in Table 1. While the data broadly suggest that there are no IL-1α binding sites that do not also bind IL-1β or vice versa, it is striking that $K_i$ values measured against IL-1α are, in general, higher than those measured against IL-1β (Table 1). For the CB23, however, quantitative analysis of the inhibition data shows that all four data sets are compatible with a maximum inhibition level of 100%, suggesting that at the concentrations of $^{125}$I-IL-1α and $^{125}$I-IL-1β used, there are no sites occupied by either radioligand that do not bind both IL-1α and IL-1β. Since the two classes of the IL-1α binding expressed on CB23 cells come close in total number (1,820 ± 280) to the total IL-1β binding sites (2,400 ± 40), we propose the following model: There are $\sim 2,000$ IL-1β binding sites on the cells; $\sim 10\%$ of these bind labeled and unlabeled IL-1α with a higher affinity than the remaining $90\%$. Conversely, labeled IL-1β binds to all the receptors with an equal affinity. Thus, the different $K_i$ values obtained for IL-1α versus $^{125}$I-IL-1α compared with IL-1β versus $^{125}$I-IL-1β are accounted for by the fact that, under the conditions used (2 to 3 $\times 10^{-10}$ mol/L $^{125}$I-IL-1α and $\sim 1 \times 10^{-9}$ mol/L $^{125}$I-IL-1β), most of the $^{125}$I-IL-1α will be bound to the high affinity receptors, whereas only 10% of the bound $^{125}$I-IL-1β will be bound to these since it cannot discriminate between high and low affinity receptors and, hence, binds to these two classes at random. Given the more marked heterogeneity in the $^{125}$I-IL-1α binding isotherms than in the $^{125}$I-IL-1β, and the possibility that IL-1β became inactivated by radiolabeling, we raise the possibility that the low number of sites that bind labeled and unlabeled IL-1α with a $K_a$ of $\sim 5$ to $10 \times 10^6$ mol$^{-1}$ also bind unlabeled IL-1β with a similar $K_a$, but that labeled IL-1β fails to bind these with high affinity due to labeling damage. Restudying the cell lines with $^{125}$I-IL-1β from different sources yielded the same data, suggesting that the differences shown for $^{125}$I-IL-1α and $^{125}$I-IL-1β are not due to a particular damaged labeled preparation of $^{125}$I-IL-1β, but are characteristic of Bolton-Hunter labeled material in general. It has been previously shown that this is the only method that allows retention of any biologic activity.

Crosslinking studies. To characterize further the nature of the two receptor populations on these cell lines, affinity crosslinking experiments were performed (Fig 7). The CB23 line was chosen for this since these cells express the most clearly resolved populations of receptors for IL-1α (Table 1). A concentration of $1.5 \times 10^{-9}$ mol/L of $^{125}$I-IL-1α or $^{125}$I-IL-1β were used to yield approximately equal levels of binding to the two types of receptor: approximately 200 high affinity sites bound and 250 low affinity sites, as estimated from the parameters in Table 1. Analysis of the detergent extract revealed that when $^{125}$I-IL-1α alone or $^{125}$I-IL-1β
alone were used, two high M s species were detected in addition to the species at M, 17,500 (presumably 125I-IL-1α itself), and that these species are not detected when unla-
beled IL-1α or unlabeled IL-1β were added as inhibitors. This indicates that the species represent specific crosstalk of IL-1α and IL-1β to cell surface proteins. When the size of the two high M s species was estimated from the markers run in parallel, values of M, 98,000 and 87,000 were obtained for both IL-1α and IL-1β. Since 125I-IL-1/IL-1 complexes contain IL-1, these bands represent surface proteins of M, 80,500 and 69,500. Previous reports have shown that T cells and fibroblasts possess an IL-1 receptor with a size close to M, 80,000,20,24,25 while human B cells have receptors with a size in the range of M, 60,000 to 68,000.9,12 Thus, our data suggest that CB23 cells coexpress both types of receptors. Crosslinking data for PA6828M-2 and CB33 also demonstrated M, 80,500 and 69,500 as shown for the CB23 cell line (data not shown). Since the possibility was raised that the

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**Table 1. Summary of Results Obtained in Direct and Competitive Binding Experiments**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>CB23</th>
<th>PA6828M-2</th>
<th>CB33</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. R1α (sites/cell)</td>
<td>220 ± 80</td>
<td>300 ± 9</td>
<td>332 ± 48</td>
</tr>
<tr>
<td>2. K1α (mol⁻¹)</td>
<td>5.0 ± (3 x 10⁻⁶)</td>
<td>5.8 ± (0.5 x 10⁻⁶)</td>
<td>5.0 ± (1 x 10⁻⁶)</td>
</tr>
<tr>
<td>3. R2α (sites/cell)</td>
<td>1,600 ± 200</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>4. K2α (mol⁻¹)</td>
<td>1.4 ± (0.5 x 10⁻⁶)</td>
<td>1.2 ± (0.2 x 10⁻⁶)*</td>
<td>1.3 ± (0.3 x 10⁻⁶)*</td>
</tr>
<tr>
<td>5. NSBa (sites/mol⁻¹)</td>
<td>1.3 x 10⁻¹</td>
<td>1.07 x 10⁻¹</td>
<td>3.3 x 10⁻¹</td>
</tr>
<tr>
<td>6. R1β (sites/cell)</td>
<td>2.400 ± 40</td>
<td>323 ± 3</td>
<td>1.127 ± 280</td>
</tr>
<tr>
<td>7. K1β (mol⁻¹)</td>
<td>6.7 ± (0.3 x 10⁻⁶)</td>
<td>9.0 ± (0.2 x 10⁻⁶)</td>
<td>1.6 ± (0.5 x 10⁻⁶)</td>
</tr>
<tr>
<td>8. R2β (sites/cell)</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>9. K2β (mol⁻¹)</td>
<td>—</td>
<td>—</td>
<td>2.2 ± (0.7 x 10⁻¹)*</td>
</tr>
<tr>
<td>10. NSBβ (sites/mol⁻¹)</td>
<td>2.1 x 10⁻¹</td>
<td>1.4 x 10⁻¹</td>
<td>3.2 x 10⁻¹</td>
</tr>
<tr>
<td>11. Kα (vβ; mol⁻¹) (lb,%)</td>
<td>1.2 ± (0.2 x 10⁻⁶) (100)</td>
<td>8.0 ± (3.0 x 10⁻⁶) (68)</td>
<td>1.6 ± (0.9 x 10⁻⁶) (70)</td>
</tr>
<tr>
<td>12. Kα (vα; mol⁻¹) (lb,%)</td>
<td>5.2 ± (0.7 x 10⁻⁶) (100)</td>
<td>1.7 ± (0.2 x 10⁻⁶) (93)</td>
<td>6.4 ± (1.0 x 10⁻⁶) (95)</td>
</tr>
<tr>
<td>13. Kβ (vβ; mol⁻¹) (lb,%)</td>
<td>4.3 ± (0.5 x 10⁻⁶) (100)</td>
<td>5.1 ± (0.9 x 10⁻⁶) (109)</td>
<td>1.7 ± (0.5 x 10⁻⁶) (86)</td>
</tr>
<tr>
<td>14. Kβ (vα; mol⁻¹) (lb,%)</td>
<td>3.6 ± (0.3 x 10⁻⁶) (100)</td>
<td>1.1 ± (0.1 x 10⁻⁶) (96)</td>
<td>3.8 ± (0.8 x 10⁻⁶) (94)</td>
</tr>
</tbody>
</table>

The values in rows 1 through 4 and 6 through 9 are derived from the direct binding data for the two forms of IL-1 to each of the three cell lines examined. The data were analyzed by a nonlinear least squares fitting of a model of the general form: Bound = xₙ KcC/ml + KcC. Where the data for a

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**Fig 4.** Cross-competition study of IL-1α and IL-1β in CB23 cell line. Inhibition of the binding of 125I-IL-1α by unlabeled IL-1α (Δ) and by unlabeled IL-1β (Θ); inhibition of 125I-IL-1β by unlabeled IL-1/β (Δ) and by unlabeled IL-1α (Ο).

**Fig 5.** Cross-competition study of IL-1α and IL-1β in CB33 cell line. Symbols defined in Fig 4.
cells were washed, treated with crosslinker, extracted, and analyzed on SDS-PAGE. As described in Materials and Methods, M, 68,000 IL-1 binding species is associated with at least one more protein. Crosslinking experiments in our B cell lines showed patterns different from those reported for either T or B cells. We demonstrate that both M, 80,000 and M, 69,500 receptor species are expressed on these cells. The higher M, species has previously been described on T cells and fibroblasts but not on B cells. Rather, several recent reports place the size of the B cell IL-1 receptor in the range of the lower M, species we observed (60,000 to 69,000). Thus, our data suggest that the B cell line CB23 expresses the IL-1 receptor previously suggested to be T lineage specific, in addition to the B cell lineage IL-1 receptor subtype. These data are in agreement with those of Bensimon et al who recently demonstrated the expression of two distinct affinity binding sites for IL-1α on the EBV-transformed cell line, 3B6. In crosslinking, however, they detected only the lower species of the IL-1 receptor (M, 68,000 to 72,000).

Recent data from our laboratory also showed that IL-1 receptors were expressed in 12 of 16 EBV-positive cell lines, but in only 1 of 8 EBV-negative cell lines. Thus, it is possible that some aspect of EBV transformation is responsible for the “inappropriate” expression of the T cell type receptor in these cells. However, the exact mechanism underlying these phenomena is at this point unknown but presumably will be answered once genomic clones for the two types of the IL-1 receptors are identified, and the nature of the promoter and enhancer regions in the genes are defined.

DISCUSSION

The existence of two distantly related species of IL-1, IL-1α and IL-1β, each of which is initially translated as an M, 30,000 protein and subsequently processed to an M, of 17,500, raises the issue which of these four forms of IL-1 can bind to cells, and whether those forms that do bind interact with different receptors or a common receptor. Dower et al showed that binding of radiolabeled IL-1β to murine T lymphoma cells can be blocked completely by unlabeled IL-1α; conversely, radiolabeled IL-1α binding can be totally blocked by IL-1β. A similar observation was made for human EBV-transformed human B cells by Matsushima et al, who showed that unlabeled human IL-1α blocked 125I-IL-1β binding. This property, ie, that the two forms of IL-1 share a common receptor, was also reported for other hormones, such as tumor necrosis factor (TNF) and platelet derived growth factor (PDGF), raising the question as to what advantage is to be gained from the existence of two different molecules with an identical spectrum of activities. Recent data already indicate that two populations of PDGF receptors that recognize different isoforms of PDGF exist, and that receptor subsets for TNFα and TNFβ might exist as well (unpublished data). Similarly, several previous reports point to the possible existence of IL-1 receptor subsets, and to differences in the binding and functional activities of IL-1α and IL-1β. These reports were further corroborated by crosslinking studies that demonstrated IL-1 receptors with M, species of 135,000, 180,000, and ~200,000, suggesting that the major M, 82,000 IL-1 binding species is associated with at least one more protein. Crosslinking experiments in our B cell lines showed patterns different from those reported for either T or B cells. We demonstrate that both M, 80,000 and M, 69,500 receptor species are expressed on these cells. The higher M, species has previously been described on T cells and fibroblasts but not on B cells. Rather, several recent reports place the size of the B cell IL-1 receptor in the range of the lower M, species we observed (60,000 to 69,000). Thus, our data suggest that the B cell line CB23 expresses the IL-1 receptor previously suggested to be T lineage specific, in addition to the B cell lineage IL-1 receptor subtype. These data are in agreement with those of Bensimon et al who recently demonstrated the expression of two distinct affinity binding sites for IL-1α on the EBV-transformed cell line, 3B6. In crosslinking, however, they detected only the lower species of the IL-1 receptor (M, 68,000 to 72,000).

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This work is currently feasible regarding the recent breakthroughs in the field of IL-1 receptor. 11,12,13,14 Chizzonite et al. 13 reported that the high affinity IL-1 receptors expressed by mouse pre-B cells, macrophages, and bone marrow granulocytes are different gene products than the previously characterized receptors expressed by mouse T cells and fibroblasts. They have classified these two types of high affinity IL-1 receptors as the T cell/fibroblast-type and B cell/macrophage-type receptors, respectively. Their conclusions were supported by the following data: (1) Radiolabeled IL-1 binds and crosslinks to different proteins on the cell surfaces of the two classes of cells. (2) Monoclonal and polyclonal anti-IL-1 receptor antibodies blocked IL-1 binding and immunoprecipitated the T cell/fibroblast-type receptor, but did not bind to the B cell/macrophage-type receptor. (3) Identical IL-1 receptor mRNA was detected in T cells and fibroblasts but not in pre-B cells and macrophages. Similarly, Bomsztyk et al. 11 showed that the IL-1 receptors expressed on the murine pre-B cell line 70Z/3 and the T cell lymphoma cell line EL-46.1C10 are structurally different. Evidence for these differences were provided by the following data: (1) A monoclonal antibody against the IL-1 receptor cloned from EL-46.1C10 did not recognize the IL-1 receptor expressed on 70Z/3 cells. (2) The molecular mass of the IL-1 receptor (deduced from affinity crosslinking) on 70Z/3 was lower than the mass deduced for the IL-1 receptor on EL-46.1C10 cells. (3) The phorbol ester phorbol myristate acetate (PMA) was an equally potent activator of protein kinase C in 70Z/3 and EL-46.1C10 cells, but it only downregulated IL-1 receptors on 70Z/3 and not on EL-46.1C10 cells. (4) Inability to detect mRNA that corresponded to the T cell IL-1 receptor in 70Z/3 cells either by Northern blot hybridization or by cDNA library screening.

Since the mouse IL-1 receptor is very closely related to the human IL-1 receptor, both in sequence and in overall organization, it is most likely that the differences in murine IL-1 receptors described in these studies 11,12 will also correspond to IL-1 receptors expressed on human B and T lymphocytes. Recently, Sims et al. 14 who cloned and sequenced IL-1 receptors from human T cells and human dermal fibroblasts, determined that these receptors’ nucleotide sequences are identical. With the availability of this new information on human IL-1 receptors present on T cells, 11,12 studies are currently underway to determine whether the IL-1 receptors expressed on human B cells are different. Even if such differences in the structure of IL-1 receptors expressed on human B and T cells are detected, their biologic implications are presently unknown. Ostrowski et al. 15 recently demonstrated that IL-1 induced transient translocation of protein kinase C to membranes in 70Z/3 cells. This effect was not observed when an IL-1--dependent T cell line was exposed to IL-1. 16 This discrepancy may reflect triggering of different IL-1 receptors that appear to exist in murine T and B lymphocytes. Whether this applies to IL-1 receptors expressed on human B and T lymphocytes is yet to be determined.

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Human B cells express two types of interleukin-1 receptors

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