Integrated Pathways for Neutrophil Recruitment and Inflammation in Leprosy

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Neutrophil recruitment is pivotal to the host defense against microbial infection, but it also contributes to the immunopathology of disease. We investigated the mechanism of neutrophil recruitment in human infectious disease by means of bioinformatic pathways analysis of the gene expression profiles in the skin lesions of leprosy. In erythema nodosum leprosum (ENL), which occurs in patients with lepromatous leprosy and is characterized by neutrophil infiltration in lesions, the most overrepresented biological functional group was cell movement, including E-selectin, which was coordinately regulated with interleukin 1β (IL-1β). In vitro activation of Toll-like receptor 2 (TLR2), up-regulated in ENL lesions, triggered induction of IL-1β, which together with interferon-γ induced E-selectin expression on and neutrophil adhesion to endothelial cells. Thalidomide, an effective treatment for ENL, inhibited this neutrophil recruitment pathway. The gene expression profile of ENL lesions comprised an integrated pathway of TLR2 and Fc receptor activation, neutrophil migration, and inflammation, providing insight into mechanisms of neutrophil recruitment in human infectious disease.

The recruitment of neutrophils to the site of infection is required for the host defense against many bacterial infections [1–3]. The recruitment of neutrophils can also result in tissue injury. Neutrophil-mediated tissue injury occurs in immune complex diseases in which the chronic deposition of immune complexes in tissues results in inflammation. These infiltrating neutrophils are thought to release toxic oxygen intermediates and proteases, leading to local tissue damage [4].

In humans, the mechanisms of neutrophil recruitment to the site of infection can be investigated using leprosy as a model. The disease forms a spectrum of clinical manifestations that correlate with the immune response to the pathogen, Mycobacterium leprae. This spectrum is dynamic, with patients developing immune reactions, and in particular, in patients with the disseminated form, lepromatous leprosy, a reaction known as erythema nodosum leprosum (ENL) [5, 6] is frequent, being observed in up to 50% of patients with lepromatous leprosy receiving antimicrobial therapy [7]. ENL is characterized by the eruption of erythematous, painful nodules and other systemic manifestations of tissue injury [8]. In regard to histology, neutrophils are the signature cells in ENL lesions, with granulomas present to an extent similar to that observed in lepromatous leprosy lesions [9]. Immune complex deposition is
thought to contribute to the pathogenesis of ENL, as evidenced by granular deposits of immunoglobulin and complement in a perivascular [10] and extravascular [11] distribution, detection of immune complexes in vessel walls, and evidence of damaged endothelial cells [6]. Given that a key clinical difference between ENL and lepromatous leprosy is the characteristic infiltration of neutrophils in ENL lesions, a major goal of this study was to investigate the mechanisms of neutrophil recruitment at the site of disease.

**METHODS**

**Clinical specimens.** The acquisition of all specimens was approved by the committees on investigations involving human subjects of the University of California, Los Angeles. For all procedures, informed consent was obtained. Clinical classification of patients with symptomatic *M. leprae* infection was performed according to the criteria of Ridley and Jopling [12]. Skin biopsy specimens were embedded in optimal cutting tissue compound (Sakura Finetek USA) and snap-frozen in liquid nitrogen.

**Microarray analysis.** RNA was isolated from skin biopsy specimens and used for microarray analysis as described elsewhere [13], with the following modifications: Messenger RNA (mRNA) was further purified using the RNeasy Micro kit (Qiagen). To generate complementary RNA probes, 100 ng of total RNA was doubly amplified using the RiboAmp RNA amplification kit (Arcturus Bioscience). The probes were purified and fragmented and then were hybridized to the U133A Plus 2.0 GeneChip (Affymetrix). The raw gene expression data for the patients with leprosy analyzed in this study will be available online through the National Center for Biotechnology Information Gene Expression Omnibus Web site (http://www.ncbi.nlm.nih.gov/geo/).

**Gene expression analyses.** Hierarchical clustering analysis [14] using 3158 probe sets with significant variation across the samples (standard deviation, >5000; coefficient of variation [standard deviation/mean], >0.3) was performed. The probe sets used were also required to have Affymetrix present calls in at least half of the experiments. Cluster diagrams were generated using the Cluster (version 2002) and TreeView (version 1.60) software programs (available at http://rana.lbl.gov). Permutation analysis was used to determine the statistical significance of our results. We systematically considered 1000 random patient groupings and determined the frequency at which a grouping yielded a result equal to or better than that of the defined ENL–lepromatous leprosy grouping, as described elsewhere [15]. For gene expression–based prediction, we used the weighted gene voting algorithm of Golub et al [16] with minor modifications, as described elsewhere [15].

To identify the most informative set of differentially expressed genes between the 2 leprosy subclasses, each gene was ranked by the probability that the means of its expression values were statistically distinct between the patients with ENL and those with lepromatous leprosy, using Student’s *t* test. The fold change in mean expression of each gene was also calculated between the 2 groups and used as a secondary ranking. We focused our attention on genes that met our designated criteria: a fold change of ≥2 and *P* ≤ .05, as calculated using Ingenuity Pathways Analysis software (version 6.0; Ingenuity Systems).

The Pearson correlation between the expression of E-selectin and the expression of all other genes on the microarray from all samples was calculated. A threshold of *R*² = 0.5 was used to obtain a list of genes that have a linear relationship in expression with E-selectin.

**Functional group and canonical pathway analyses.** The functional group and canonical pathway analyses were generated through the use of Ingenuity Pathways Analysis software. Probe sets that were increased in expression in ENL compared with lepromatous leprosy for which the fold change was >2.0 and *P* < .05 were included in the analysis. The Fisher exact test was used to calculate a *P* value to determine the probability that each biological function was due to chance alone.

**Immunohistochemical and flow cytometry studies.** Immunoperoxidase labeling of cryostat sections was performed as described elsewhere [17]. The primary monoclonal antibodies used were C126C10B7 (anti–E-selectin; Biosource) and F8/86 (anti–von Willebrand factor; DakoCytomation). The antibodies used in flow cytometry included 68-5H11 (anti–E-selectin; BD Pharmingen) and MEM-111 (anti–intercellular adhesion molecule 1 [ICAM-1]; Caltag).

**Stimulation of peripheral blood monocytes and endothelial cells.** Peripheral blood mononuclear cells were purified by Ficoll-Hypaque (Pharmacia Biotech AB) gradient centrifugation followed by percoll (Amersham Biosciences) gradient separation to obtain monocytes (purity, 90%). Cells were stimulated in triplicate with plate-bound human immunoglobulin (hIgG) (Equitech-Bio) or the Toll-like receptor 2/1 heterodimer (TLR2/1) agonist, a lipopeptide derived from the *M. leprae* 19-kDa antigen. Interleukin 1β (IL-1β) and interleukin 8 (IL-8) were measured by enzyme-linked immunosorbent assay from cultured supernatant after overnight stimulation. Human umbilical vein endothelial cells (HUVECs) (Cambrex BioScience) at passage 3–6 were used for all experiments. HUVECs were stimulated overnight with IL-1β (1 ng/mL) and interferon γ (IFN-γ) (10 ng/mL). Cells were analyzed by flow cytometry or used in neutrophil-binding assays. Thalidomide enantiomers (Sigma) in dimethyl sulfoxide (Sigma) were added at 50 μg/mL. Statistical analysis was performed using paired Student’s *t* test.

**Neutrophil-binding assays.** Neutrophils were prepared as described elsewhere [18]. Neutrophil-binding experiments were performed using the CytoSelect leukocyte-endothelium adhe-
Figure 1.  

A, Unsupervised data analysis separating leprosy lesion samples into clinically relevant subclasses on the basis of gene expression patterns. Hierarchical clustering analysis divides 7 lepromatous leprosy and 6 erythema nodosum leprosum (ENL) skin lesion samples into 2 distinct groups that cluster on separate branches of a dendrogram. There are 3158 probe sets represented in this diagram.  

B, Permutation analysis of the microarray data, revealing that only <0.1% of the permutated groupings manifest more distinction in gene expression than does the defined ENL–lepromatous leprosy patient grouping. The cumulative numbers of probe sets (y-axis) with Student's t test–calculated P values less than various threshold levels (x-axis) were calculated for the clinically relevant ENL–lepromatous leprosy grouping and plotted (black line). One thousand randomly permutated groupings were also generated and tested. We plotted the mean (red line), tenth percentile (green line), first percentile (blue line), and one-tenth percentile (yellow line) number of probe sets with differential expression below a given P value among the permutated groupings and compared these with the correct ENL–lepromatous leprosy grouping. Compared with the 0.1% confidence level, the ENL–lepromatous leprosy grouping generally has more differentially expressed probe sets with P values below the indicated threshold, indicating that the ENL–lepromatous leprosy grouping is statistically significant.  

C, Prediction accuracy determined using leave-one-out cross-validation and weighted gene voting. Our prediction algorithm used the ENL–lepromatous leprosy grouping to correctly assign the subclasses of 12 of 13 samples with high confidence (prediction strength >0.4). The arrow indicates the ENL–lepromatous leprosy grouping, and the number above each bar indicates the number of random groups that correctly assigned the number of samples on the x-axis. L-lep, lepromatous leprosy.

RESULTS

Differential gene expression profiles comparing ENL with lepromatous leprosy. The gene expression profiles of the skin lesions of patients with ENL compared with those of patients with lepromatous leprosy were investigated using Affymetrix microarrays, to identify the gene families and/or pathways involved in disease pathogenesis. Skin biopsy specimens were obtained from 6 patients with ENL and 7 patients with lepromatous leprosy. Hierarchical clustering analysis of the gene expression data separated the samples into 2 distinct groups that are consistent with the patients’ clinical and histopathological diagnoses (Figure 1A). Therefore, the lesions from patients with ENL have gene expression profiles that are distinct from those of the lesions from patients with lepromatous leprosy.

To determine whether the observed differences in gene expression between the patients with ENL and those with lepromatous leprosy were statistically significant, a permutation analysis was performed in which the number of probe sets
Figure 2. Enriched pathways in erythema nodosum leprosum (ENL) versus those in lepromatous leprosy. The functional and canonical pathways analyses were generated through the use of Ingenuity Pathways Analysis software (version 6.0; Ingenuity Systems). A, Top 20 biological functions in ENL compared with lepromatous leprosy, ranked by their $P$ values (x-axis). B, All statistically significant canonical pathways represented by the differentially expressed genes, ranked by their $P$ values (x-axis). The Fisher exact test was used to calculate each $P$ value to determine the probability that each pathway represented by the expression data was due to chance alone. IL-6, interleukin 6; L-lep, lepromatous leprosy; PI3K/AKT, phosphoinositide 3-kinases/v-akt murine thymoma viral oncogene homolog 1; VEGF, vascular endothelial growth factor.

below each $P$ value threshold in the clinically relevant ENL or lepromatous leprosy grouping was compared with 1000 random groupings (permutated groups). This analysis showed that $<0.1\%$ of the permutated groupings displayed more differential expression than did the correct ENL–lepromatous leprosy grouping (Figure 1B). The cumulative number of probe sets ($y$-axis) with Student's $t$ test $P$ values less than various threshold levels ($x$-axis) was calculated for the actual clinically diagnosed ENL–lepromatous leprosy grouping and plotted. For example, at $P = .01$ the ENL–lepromatous leprosy grouping had many
more differentially expressed probe sets (37,467) than did both the mean (3915) and the top 0.1% (25,963) of the permutated groupings. This result established that, despite the relatively small number of samples examined in this study and the cellular heterogeneity of the specimens, the observed differences in gene expression between patients with ENL and those with lepromatous leprosy were not likely to have resulted by chance.

To evaluate whether gene expression profiles were sufficiently robust to correctly assign the subclasses of unknown samples, leave-one-out cross-validation analysis was performed [16]. Using the clinically defined ENL versus lepromatous leprosy patient grouping, the algorithm predicted the classes of all 13 samples correctly (accuracy, 100%; 12 of 13 samples had high confidence [prediction strength, >0.4]). This result is statistically significant, because only 1 of 1000 (0.1%) random patient groupings performed better than the clinically defined ENL-lepromatous leprosy grouping at classifying the withheld sample (Figure 1C). Taken together, the results from both the unsu-
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Figure 4. A, Correlation of E-selectin expression with interleukin 1β (IL-1β) and interleukin 1 receptor type 1 (IL-1R1). Shown are the \( R^2 \) values (Pearson correlation) between the expression of E-selectin and the expression of IL-1β and IL-1R1. B, In vivo E-selectin and von Willebrand factor protein expression in lepromatous leprosy skin lesions, compared with that in erythema nodosum leprosum (ENL) skin lesions. Thin (4 μm) sections of leprosy biopsy samples were incubated with anti–E-selectin or with anti–von Willebrand factor and stained secondarily by an immunoperoxidase method followed by counterstaining with hematoxylin. Each bar denotes 100 μm. The isotype controls were negative. The findings shown are representative of 5 patients in each group. AU, arbitrary units; L-lep, lepromatous leprosy.

Differentially expressed functional groups in ENL skin lesions versus lepromatous leprosy skin lesions. To detect the gene sets or biological pathways that are overrepresented in ENL compared with lepromatous leprosy and that might be particularly relevant to disease pathogenesis, we compared the gene expression profile of ENL skin lesions with that of lepromatous leprosy skin lesions by using knowledge-guided bioinformatic analysis that incorporated data on likely biological functions, including gene ontology information and regulatory data (Ingenuity Systems). This analysis identified 57 functional groups \( (P < .05) \) and 17 canonical pathways \( (P < .05) \) (Figure 2). The striking finding was that the top functional pathway identified in ENL lesions versus lepromatous leprosy lesions was cell movement, which was composed of 188 genes \( (P = 1.98 \times 10^{-13}) \) (Figure 2A) and included genes that are involved in recruitment of neutrophils, the “signature” cell in ENL lesions. Functional pathway analysis using the Database for Annotation, Visualization, and Integrated Discovery \( [19] \) yielded consistent results, because the top pathways all involved cell adhesion (data not shown). In addition, the top canonical pathway identified was eicosanoid signaling (Figure 2B). Use of the Benjamini-Hochberg method to correct for multiple testing yielded similar functional groups (data not shown); however, this highly stringent method led to no statistically significant canonical pathways. Because the eicosanoid signaling pathway gene members were comparatively increased in expression, including both the enzymes for ligands and their corresponding receptors (Figure 7), in comparing ENL lesions with lepromatous leprosy lesions, we decided to use a less stringent approach that was more biologically relevant.

The cell movement list of genes that was differentially expressed in ENL lesions was further investigated by limiting the gene set to those functional groups involved in neutrophil recruitment, which identified 25 genes (Figure 3A). These genes were also characterized according to cellular location of the proteins encoded by each of the genes on the basis of the supervised and the supervised prediction algorithms demonstrate that ENL lesions and lepromatous leprosy lesions have distinct gene expression profiles.
Figure 5. Effect of Fc receptor or Toll-like receptor 2/1 heterodimer (TLR2/1) activation of peripheral blood monocytes on interleukin 1β (IL-1β) (A) and interleukin 8 (IL-8) (B) secretion. Percolled monocytes from healthy donors were stimulated overnight with a lipopetide known to stimulate TLR2/1 or plate-bound human immunoglobulin G (hlgG) in medium or in (+)-(R)-thalidomide (Thal R) or (-)-(S)-thalidomide (Thal S) enantiomers. IL-1β and IL-8 were measured from cultured supernatant by enzyme-linked immunosorbent assay. Shown are mean values for triplicate cultures; error bars indicate standard errors of the mean. Asterisks denote differences between medium- and thalidomide-treated cells with *P < .05 paired Student’s t test. TLR2/1L, Toll-like receptor 2/1 heterodimer ligand.

Ingenuity pathways knowledge base (Figure 3B). Examination of the genes encoding proteins expressed on the cell surface that are involved in neutrophil recruitment revealed the differential up-regulation in ENL lesions of the selectin family of adhesion molecules, including E-selectin (fold change, 3.5; P < .05) and P-selectin (fold change, 2.8; P < .001). E-selectin is a key molecule involved in neutrophil binding to endothelium, an initial step in neutrophil recruitment [20, 21] that also contributes to the recruitment of other leukocytes. Analysis revealed that the E-selectin ligands, E-selectin ligand 1 (fold change, 2.8; P < .001), CD44 (fold change, 1.7; P < .05), and P-selectin glycoprotein ligand 1 (fold change, 1.7; P < .05), were also expressed at higher levels in ENL, although for 2 molecules there was a <2-fold change. In summary, the bioinformatics analysis of ENL lesions according to biological pathways revealed the up-regulation of cell movement genes, including E-selectin and its ligands, key molecules that mediate neutrophil binding to endothelial cells.

Regulation of E-selectin expression. To identify the inflammatory mediators that contribute to E-selectin expression in ENL lesions, we queried which genes were coordinately regulated with E-selectin expression in individual lesions. From the ∼3000 genes identified to be coordinately regulated with E-selectin, 49 genes were identified as part of the cell movement biological function related to neutrophil recruitment. Five genes were identified by pathway analysis to have a first-level interaction with E-selectin, of which only IL-1β up-regulates E-selectin (R² = 0.65). In addition, the expression of IL-1 receptor type 1 (IL-1R1) correlated with E-selectin expression (R² = 0.54) (Figure 4A). Although IL-1β had a 5-fold increase in ENL lesions compared with lepromatous leprosy lesions, individual variability was high; therefore, the direct comparison between lesions did not achieve statistical significance. However, relative increases in IL-1R1 in ENL lesions did achieve significance (2 different probe sets [fold change, 1.6 and 2.2; P < .001]). In addition to increased expression of IL-1R1 in ENL lesions compared with lepromatous leprosy lesions, other IL-1R1 family members and signaling molecules—such as Toll-like receptor 2 (TLR2) (fold change, 1.9; P < .05), myeloid differentiation response gene 88 (fold change, 1.5; P < .05), and
interleukin 1 (IL-1) receptor–associated kinase 1 (fold change, 1.9; \( P < .01 \))—were also increased, although these molecules had a \(<2\)-fold change. These data identify IL-1\( \beta \) and TLR2 as potential regulators of E-selectin expression on endothelial cells and subsequent neutrophil binding.

E-selectin expression in ENL skin lesions versus lepromatous leprosy skin lesions. To examine whether E-selectin protein expression reflects the differential expression of mRNA in ENL versus lepromatous leprosy, immunohistochemical analysis was performed on skin biopsy specimens from 5 patients with ENL and 5 patients with lepromatous leprosy (Figure 4B). E-selectin was expressed in a vascular pattern and at higher levels in ENL skin lesions (20%–80% of vessels) than in lepromatous leprosy skin lesions (0%–20% of vessels). The low or absent expression of E-selectin in lepromatous leprosy skin lesions was not the result of a decreased number of blood vessels, because serial sections were also labeled for an endothelial cell marker, von Willebrand factor (Figure 4B).

Effect of thalidomide on IL-1\( \beta \) production, IL-1\( \beta \)–induced E-selectin expression, and neutrophil binding. A characteristic feature of ENL is the dramatic clinical response to treatment with thalidomide, accompanied by a prompt reduction

Figure 6. A, Effect of interferon \( \gamma \) (IFN-\( \gamma \)) on interleukin 1\( \beta \) (IL-1\( \beta \)) induction of E-selectin expression on human umbilical vein endothelial cells (HUVECs). B, Effect of thalidomide on E-selectin and intercellular adhesion molecule 1 (ICAM-1) expression by HUVECs induced by IL-1\( \beta \) and IFN-\( \gamma \). C, Neutrophil binding to HUVECs stimulated with IL-1\( \beta \) and IFN-\( \gamma \) in the presence or absence of thalidomide. Data shown are a compilation of 3 independent experiments. The statistical analysis was performed using paired Student’s \( t \) test. MFI, mean fluorescent intensity; PMN, polymorphonuclear leukocytes.
in the number of neutrophils in lesions [22, 23]. Given that thalidomide has been shown to inhibit induction of proinflammatory cytokines [24–26], we hypothesized that thalidomide might also inhibit the ability of immune complexes to trigger IL-1β gene expression from monocytes. In these experiments, immune complex induction of IL-1β was modeled by using immobilized or plate-bound hlgG to activate Fc receptors, triggering monocytes to release IL-1β (Figure 5A). The racemic enantiomers of thalidomide were tested separately for their effect on IL-1β production, because it has been suggested that the (−)-(S) and (+)-(R) forms may have different effects [27]. Both enantiomers diminished the ability of plate-bound hlgG to induce IL-1β by 20%–40% (Figure 5A).

Given that TLR2 was also noted to be comparatively increased in expression in ENL lesions compared with lepromatous leprosy lesions, we also tested the ability of thalidomide to inhibit TLR2 induction of IL-1β (Figure 5A). A lipopeptide derived from M. leprae was used as the TLR2 ligand [28]. Thalidomide diminished TLR2–induced monocyte production of IL-1β by 50%–80%, whereas production of IL-8 was not affected, consistent with Oliveira et al [29] (Figure 5B). Thus, the production of IL-1β in Fc receptor (FcR)– or Toll-like receptor (TLR)–induced monocytes is partially inhibited by thalidomide.

Regulation of E-selectin expression on endothelial cells is known to be mediated by various cytokines, including IL-1β, interleukin 6 (IL-6), tumor necrosis factor α (TNF-α), and IFN-γ [30]. Furthermore, IL-1 acts directly on cultured HUVECs [31], rather than on leukocytes [32], to increase the adhesiveness of the endothelial cell surface for neutrophils. HUVECs cultured overnight with IL-1β showed a dose-responsive induction of E-selectin expression (Figure 6A). Because subcutaneous injections of IFN-γ in patients with lepromatous leprosy have been reported to induce ENL [33] and because IFN-γ is known to enhance the effects of IL-1β on E-selectin expression [30] in a dose-responsive manner (Figure 6A), we tested the ability of thalidomide to inhibit the induction of E-selectin by IL-1β and IFN-γ (Figure 6B). IFN-γ alone did not induce E-selectin expression (mean fluorescent intensity [MFI] for untreated patients, 14.3; and MFI for patients treated with highest dose of IFN-γ, 15.6). Both enantiomers of thalidomide inhibited the ability of IL-1β and IFN-γ to induce E-selectin expression by 35%–47%, whereas induction of ICAM-1 (Figure 6B) and cell viability were not affected (data not shown). P-selectin expression was not induced by IL-1β and IFN-γ (MFI for medium-treated HUVECs, 6.4; MFI for IL-1β– and IFN-γ–treated HUVECs, 5.4).

To test whether the effect of thalidomide on endothelial cell expression of E-selectin was functionally relevant, the effect of thalidomide on the ability of the HUVECs to bind to neutrophils was examined (Figure 6C). Although all cytokine-treated HUVECs bound to more neutrophils than did those treated with medium alone, those treated with IL-1β and IFN-γ bound to >3-fold more neutrophils than did those cultured with medium. Furthermore, the addition of thalidomide resulted in 23%–25% diminished neutrophil binding to HUVECs stimulated with IL-1β and IFN-γ. Taken together, these data suggest a novel mechanism of action for thalidomide in inhibiting IL-1β release, IL-1β induction of E-selectin, and subsequent neutrophil binding to endothelial cells.

**DISCUSSION**

To investigate the mechanisms of neutrophil recruitment and subsequent tissue injury, we determined the gene expression profile of the skin lesions that characterize ENL, a reaction that occurs in patients with the lepromatous form of leprosy. Using bioinformatics analysis of immune response pathways in combination with in situ analysis of lesions and in vitro functional experiments, an integrated pathway was identified. Major aspects of this pathway include the following: (1) FcR or TLR2 induction of IL-1β release; (2) endothelial activation, including the up-regulation of E-selectin and subsequent neutrophil binding; and (3) up-regulation of inflammatory mediators associated with both neutrophils and monocytes/macrophages. Thalidomide, a highly effective agent used in the treatment of ENL that is known to reduce neutrophil infiltration in lesions, targeted individual events of this inflammatory pathway. Taken together, these data provide evidence that a local inflammatory mechanism linking IL-1β, E-selectin, and neutrophil recruitment is part of a clinically relevant integrated pathway of tissue injury.

A key mechanism by which circulating neutrophils are recruited to disease sites involves the up-regulation of E-selectin on endothelial cells, which mediates the initial adhesion and rolling required for neutrophil binding and tethering [20, 34]. Our data suggest that macrophages may produce IL-1β that contributes to up-regulated E-selectin expression in ENL lesions, given that IL-1β was correlated with E-selectin expression in lesions. Importantly, we identified 2 distinct clinically relevant mechanisms of IL-1β induction in human disease. First, TLR2 activation of monocytes induced IL-1β release, which is relevant given that ENL develops during the initial phase of antimicrobial therapy in which bacterial breakdown is documented. Second, FcR activation of monocytes triggered the expression of IL-1β, consistent with the deposition of immune complexes containing bacterial breakdown products in lesions. The ability of IFN-γ to enhance the ability of IL-1β induction of E-selectin [30] and subsequent neutrophil binding is noteworthy, given the clinical observation that 60% of patients with lepromatous leprosy who received subcutaneous injections of IFN-γ developed ENL [33]. These observations suggest that both IL-1β and IFN-γ play a role in the induction of E-selectin...
Figure 7. Gene program of neutrophil recruitment and tissue injury, based on gene expression and in vitro data. Molecules in black text represent those added to the pathway on the basis of microarray data, whereas molecules and genes in blue text have supportive immunohistochemical and/or in vitro data. Immune complexes and/or mycobacterial components may activate Fc receptors (FcRs) and Toll-like receptors (TLRs) to induce proinflammatory cytokines such as interleukin 8 (IL-8) and interleukin 1β (IL-1β). IL-1β in combination with interferon γ (IFN-γ) induce E-selectin expression on endothelial cells, resulting in the first step of neutrophil recruitment, adhesion, and rolling along the endothelial wall. These steps are inhibited by thalidomide. Neutrophil interaction with intercellular adhesion molecule 1 (ICAM-1) as well as with other integrin-mediated activators results in arrest and activation. Glycosaminoglycans (such as heparan sulfate) and extracellular proteins (such as plasminogen activator inhibitor 1) promote the presentation of chemokines (such as IL-8) on the surface of the endothelium to mediate neutrophil activation and chemotaxis, inhibited by secretory component. As part of the innate immune response to microbial infection, the local recruitment of neutrophils may lead to tissue injury as host defense pathways are executed.

as part of the pathogenesis of ENL. Although the neutrophil-recruiting leukotriene B4 synthetic pathway and its receptor were comparatively increased in ENL lesions (Figure 7), classic neutrophil chemokines (eg, CXCL1–CXCL3 and CXCL5–CXCL8) were not.

The clinical relevance of the pathway FcR/TLR–IL-1β→E-selectin→neutrophil recruitment in the pathogenesis of tissue injury in leprosy was investigated in the context of the drug thalidomide, a particularly efficacious intervention in the management of ENL [35] that is known to decrease neutrophil infiltration in disease lesions [23]. Our data indicate that thalidomide can modulate neutrophil-mediated injury by diminishing FcR- and TLR2/1-induced IL-1β release, as well as by moderately decreasing the ability of IL-1β and IFN-γ to induce E-selectin and subsequent neutrophil adherence to endothelial cells. Thalidomide was found to down-regulate IL-1β in models of burn injury, pancreatitis, and lipopolysaccharide (LPS)-induced uveitis [25, 36, 37]. The finding that thalidomide targets
neutrophil recruitment is possibly relevant to the mechanism of action of thalidomide in the treatment of recurrent apthous stomatitis and Behçet syndrome [38, 39]. The ability of thalidomide to target the FCer/TLR−IL-1β→E-selectin→neutrophil recruitment pathway complements studies indicating that thalidomide plays a role in inhibiting neutrophil and monocyte phagocytosis, neutrophil chemotaxis, and TNF-α−induced adhesion molecules, as well as TNF-α− and LPS-induced IL-1β production by monocytes [40–44]. TNF-α expression was not comparatively increased in ENL skin lesions (data not shown). These findings warrant further serial studies of patient biopsy specimens to investigate the pathways altered by thalidomide at the site of disease.

Analyzing the gene expression profiles of disease lesions according to biological pathways provided an unbiased method of identifying mechanisms of pathogen-induced neutrophil accumulation and its inflammatory consequences. This contrasts with the “standard” supervised approach of evaluating individual genes by key identifiers. Thus, it was possible to integrate a large number of differentially expressed genes into biologically meaningful pathways. These genes, along with those coordinately up-regulated with E-selectin, were incorporated into a speculative model of neutrophil-mediated tissue injury that included genes involved in neutrophil chemotaxis, migration, infiltration, degranulation, and oxidative burst (Figure 7). In addition, the results of the pathways analysis, including the top functional groups and the canonical pathways increased in ENL compared with lepromatous leprosy, indicate other inflammatory pathways that may also contribute to the pathogenesis of ENL. For example, the genes B4GALT1 and FUT7, which are part of the cell movement functional group, are involved in glycosylation of selectins or their ligands to mediate neutrophil recruitment [45, 46]. The second most represented canonical pathway, IL-6 signaling, leads to the expression of the downstream genes aromatase and TNF-α−induced protein 6, both of which are increased in ENL lesions compared with lepromatous leprosy lesions and have been reported to contribute to tissue inflammation [47, 48]. Finally, the top canonical pathway in ENL lesions, eicosanoid signaling, likely regulates tissue inflammation.

The model of immune complex–mediated tissue injury is relevant to the recruitment of neutrophils as part of the innate immune response to control bacterial infections, as suggested in animal models of infections [2, 3, 49]. The neutrophilic abscess formed in the classical response to Staphylococcus aureus infection also requires IL-1 [2]. Although neutrophil activation is important for host defense, including the transfer of granules containing antimicrobial peptides to macrophages in mycobacterial infection [18], it also contributes to inflammation and tissue injury. The combination of bioinformatics pathways analysis with in situ and in vitro approaches provides new insight into the disease pathogenesis and novel targets for disease intervention in managing patients with immune complex–mediated diseases.

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