Immune cell transcriptome datasets reveal novel leukocyte subset–specific genes and genes associated with allergic processes

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Background: The precise function of various resting and activated leukocyte subsets remains unclear. For instance, mast cells, basophils, and eosinophils play important roles in allergic inflammation but also participate in other immunologic responses. One strategy to understand leukocyte subset function is to define the expression and function of subset-restricted molecules.
Objective: To use a microarray dataset and bioinformatics strategies to identify novel leukocyte markers as well as genes associated with allergic or innate responses.
Methods: By using Affymetrix microarrays, we generated an immune transcriptome dataset composed of gene profiles from all of the major leukocyte subsets, including rare enigmatic subsets such as mast cells, basophils, and plasma cells. We also assessed whether analysis of genes expressed commonly by certain groups of leukocytes, such as allergic leukocytes, might identify genes associated with particular responses.
Results: Transcripts highly restricted to a single leukocyte subset were readily identified (>2000 subset-specific transcripts), many of which have not been associated previously with leukocyte functions. Transcripts expressed exclusively by allergy-related leukocytes revealed well known as well as novel molecules, many of which presumably contribute to allergic responses. Likewise, Nearest Neighbor Analysis of genes coexpressed with Toll-like receptors identified genes of potential relevance for innate immunity.
Conclusion: Gene profiles from all of the major human leukocyte subsets provide a powerful means to identify genes associated with single leukocyte subsets, or different types of immune response.
Clinical implications: A comprehensive dataset of gene expression profiles of human leukocytes should provide new targets or biomarkers for human inflammatory diseases.
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Key words: *Allergy, gene profiling, leukocyte-specific, Toll-like receptors*

Host responses to pathogens involve cooperation between numerous leukocyte types, each with distinct functional properties. Over the period of the past 30 years, a principle strategy used to understand leukocyte subset function has been the characterization of molecules expressed by these subsets. Since the 1980s, mAbs have been instrumental for identifying leukocyte subsets and revealing the function of molecules and cell types. Recently, however, sequencing the human genome and development of gene microarray technologies have provided new means of identifying genes expressed and regulated in leukocyte subsets. Transcriptional profiles of a range of leukocytes at various stages of differentiation and activation have been reported, including naive and activated T cells,[1] T_H1 and T_H2,[2] follicular B T_H,[3] various B-cell subsets and lymphomas,[4-6] mast cells,[7,8] eosinophils,[9,10] macrophages, dendritic cells (DCs),[11-13] and natural killer (NK) cells.[14] To date, most microarray studies have involved just a single cell type and only a limited number of variables, such as type of stimulation or stage of differentiation. In addition, gene microarrays have been used to establish signatures for different types of immune responses[15] or different aspects of cell physiology. For instance, specific sets of genes are characteristically transcribed in response to cellular activation or proliferation,[16] and signature genes have been

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Abbreviations used
DC: Dendritic cell
MACS: Magnetic-activated cell sorting
NK: Natural killer
PC: Plasma cell
PMA: Phorbol 12-myristate 13-acetate
TLR: Toll-like receptor

described for allergic airway inflammation,[17,18] innate immune responses to pathogens,[19] and numerous cancers.[5]

Leukocyte subsets express certain molecules exclusively, and these usually underlie the specific function of that subset. For instance, B cells express immunoglobulin, mast cells express mast cell tryptase, and eosinophils express eosinophil peroxidase. Similarly, many molecules are expressed by groups of leukocyte subsets, particularly those that reflect the common role of a molecule in a particular immune response. For instance, the chemokine receptor CCR7 facilitates migration of T cells, B cells, and DCs to lymphoid tissues, enabling the interaction of these cells in primary and secondary immune responses.[20,21] Similarly, molecules such as IL-4, IL-13, CCR3, and chemoattractant receptor preferentially expressed on T_H2 cells are preferentially expressed by allergic-type leukocytes and are strongly implicated in the pathogenesis of allergic diseases.[22-24]

We used oligonucleotide microarrays to generate gene transcription profiles for virtually all of the major human leukocyte types. This comprehensive dataset enabled us to identify leukocyte subset-restricted genes using simple bioinformatics strategies. We used this dataset to assess gene expression in groups of leukocytes and, as an example, assessed genes commonly expressed in leukocytes associated with allergic responses (mast cells, basophils, and eosinophils) or coexpressed with Toll-like receptors (TLRs) and, hence, likely to be involved in innate immunity. The description herein of a comprehensive and publicly available immune transcriptome dataset and established methods for data analysis should prove a valuable resource for the identification of molecules and pathways associated with different immune responses.

METHODS

Leukocyte isolation, differentiation, and activation

All experiments were approved by the Human Research Ethics Committee, St Vincent's Hospital (Sydney, Australia). Leukocyte subsets were purified from blood from healthy volunteers. Isolation of central and effector memory T cells from PBMCs,[3] and of plasma cells (PCs), naive B cells, and memory B cells that expressed IgM (nonswitched) or IgG/A/E (isotype switched) from spleen was previously described.[25-27] PC chips were run using pooled cRNA from as many as 5 donors. $CD19^+$ B cells and $CD16^+CD56^+$ NK cells were sorted from PBMCs using a FACSVantage SE DiVa (BD Biosciences, San Jose, Calif). Doublets and aggregated cells were excluded to minimize contaminating cells.

For basophil isolation, $CD3^+$ cells and $CD14^+$ cells were depleted from PBMCs by magnetic-activated cell sorting (MACS) (Miltenyi Biotec, Auburn, Calif). Resultant cells were stained with 5E8 anti-CCR3 antibody and labeled with Cy5-conjugated donkey antimouse IgG (Jackson Laboratories, Bar Harbor, Me). $CCR3^{hi}$, forward-scatter and side-scatter low cells were collected. RNA was pooled from 2 donors for each microarray analysis.

Macrophages and DCs were differentiated from peripheral blood monocytes isolated using CD14 positive selection by MACS. Macrophages were differentiated in the presence of 500 U/mL GM-CSF (BD Biosciences) for 11 days and then activated for 4 hours with 100 ng/mL LPS (Sigma, St Louis, Mo). DCs were differentiated in the presence of 800 U/mL IL-4 (BD Biosciences) and 1500 U/mL GM-CSF and cultured for 5 days. Nonadherent immature DCs were harvested after day 5, either for RNA isolation or activation by adding 100 ng/mL LPS for 48 hours. Macrophages were identified as $CD14^{hi}$/HLA-DR^{int}/$CD40^{int}$/$CD80^-$/$CD86^+$, immature DCs were identified as $CD14^-$/HLA-DR^{int}/$CD1a^+$/$CD40^+$/$CD80^-$/$CD83^-$/$CD86^+$, and mature DCs were identified as $CD14^-$/HLA-DR^{hi}/$CD1a^+$/$CD40^{hi}$/$CD80^+$/$CD83^+$/$CD86^{hi}$ by flow cytometry.

Granulocytes were isolated using a 70%/80% isotonic Percoll (Amersham, Buckinghamshire, United Kingdom) gradient.[28,29] Erythrocytes were eliminated using the Whole Blood Erythrocyte Lyzing Kit (R&D Systems, Minneapolis, Minn). Eosinophils were removed by incubation with anti-CCR3 followed by depletion using an antimouse secondary antibody conjugated to MACS beads. RNA was extracted from resting neutrophils and neutrophils stimulated for 1 hour with 100 ng/mL LPS.

Eosinophil isolation from peripheral blood granulocytes was adapted from published methods.[29] Neutrophils were depleted from the granulocyte population using anti-CD16 MACS beads. Eosinophils were then incubated with phorbol 12-myristate 13-acetate (PMA; 50 ng/mL), or with control medium for 2 hours. RNA extracted from 3 donors was then pooled for 1 microarray experiment, and a sample from a fourth donor was used for a second experiment.

Mast cells were derived from human cord blood obtained from the Sydney Cord Blood Bank and differentiated in the presence of IL-6 (BD Biosciences), IL-10 (BD Biosciences), and stem cell factor[30] (a gift from Amgen). Mature mast cells were activated by FcεRI cross-linking with human IgE anti-nitrophenyl (Serotec, Oxford, United Kingdom) followed by 2 hours with mouse antihuman IgE (Serotec) as previously described.[31]

RNA extraction and GeneChip hybridizations

HG-U133A and B GeneChips (Affymetrix, Santa Clara, Calif) were each hybridized with cRNA synthesized from RNA from single donors, except for experiments involving PCs, eosinophils, and basophils. RNA was isolated using the RNeasy Total RNA Isolation Kit (Qiagen, Chatsworth, Calif) or TRIzol reagent (Invitrogen Life Technologies, Mt Waverley, Australia). cRNA was prepared, and GeneChips were hybridized and scanned as previously described.[3]

Leukocyte signature analysis

Leukocyte-specific genes were defined as genes constitutively expressed by a particular leukocyte type and consistently absent in all other leukocyte subsets profiled. Only those genes showing similar patterns of expression in duplicate GeneChip experiments were considered for further analysis—that is, genes were considered to be present only if they were consistently detected. To increase the stringency of genes defined to be unique to a certain leukocyte type, genes present in naive and memory B cells (Good et al, Unpublished data, March 2004), γδ T cells, follicular B T_H, T_H1 and T_H2, and naive and central memory T-cell subsets[32] were subtracted from the initial

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