Neutrality, Compensation, and Negative Selection during Evolution of B-Cell Development Transcriptomes

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B cells develop in the mammalian bone marrow through a sequence of precursor stages, which can be ordered by the recombination status of their immunoglobulin loci. This developmental pathway is functionally similar between mice and man. However, whether this similarity is based on usage of the same genes is unknown. We show that large-scale gene expression patterns differ substantially between human and mouse B-cell development. Among 644 genes which were differentially expressed in 4 early stages of human B-cell development, only 48, 86, and 75 genes could be identified, which are upregulated in both human and mouse pre–BI, large pre–BI, and small pre–BII cells, respectively. A comparison of mouse B- and T-cell development reveals that gene expression patterns of early murine B- and T-cell precursors are most similar, whereas in more differentiated precursors, human and mouse B cells have a more similar gene expression profile. We conclude that large-scale differences in gene expression patterns between human and mouse B-cell precursors may stem from either selective neutrality or compensatory evolution, whereas the few similarities may stem from negative selection. Gene expression patterns are shaped by ontogenic relationships in early and by functional specialization in later stages of development.

Introduction

Mammalian B lymphocytes develop from progenitors and precursor cells in bone marrow in a sequence that can be ordered by stepwise rearrangements of their immunoglobulin gene loci (Melchers and Rolink 1999). This sequence of developmental stages is remarkably similar between mice and men, and cell surface markers have been described in both species that allow for purification of 6 cell populations that follow each other in progressive differentiation (Rolink et al. 1994; Ghia et al. 1996):

a) D1-J3 rearranged, cycling pre–BI cells (c-kit+ CD25− in mice, CD19+ CD34+ in man);
b) V1H-DµH-LJL-rearranged, cycling large pre–BII cells (c-kit−CD25+ pre-B1 cell receptor (BCR) in mice, CD19+ CD34−pre-BCR+ in man);
c) pre–BCR negative cycling large pre–BII cells;
d) V1H-DµH- and V1J-L rearranged resting small pre–BII cells (c-kit−CD25+ in mice, CD19+CD34−pre-BCR− sIgM− in man);
e) sIgM+ resting immature; and
f) sIgM− IgD+ resting mature B cells.

Similarly to B-cell development, development of T cells also occurs through a series of cellular stages and is comparable to B-cell development in several aspects: T-cell development, too, is governed by stepwise DNA recombination (of the T-cell receptor beta and alpha chains), and cellular stages that follow each other in progressive differentiation can be analyzed by expression of surface markers (CD24, CD44, CD4, and CD8, in this case). Corresponding stages between B- and T-cell development have been defined (Hoffmann et al. 2003).

This situation of relatively conserved cellular differentiation pathways is quite unique. However, whether this phenotypic and functional conservation is based on conserved gene usage is not known. Here, we analyze large-scale gene expression patterns of cells isolated ex vivo from 4 consecutive stages of human B-cell development. By comparing gene expression patterns of human B-cell development with mouse B-cell development and mouse B-cell development with mouse T-cell development, we dissect the relative impact of evolutionary forces, ontogenic relatedness, and functional specialization.

Materials and Methods

Isolation of Human B-Cell Precursors

Human heparinized bone marrow was obtained by iliac crest aspiration from 6 juvenile individuals (aged 1–12 years, 2 males and 4 females), as part of the initial workup of newly diagnosed nonhematologic malignant disease (medulloblastoma, Ewing’s Sarkoma (n = 2), chloroma, rhabdomyosarcoma, and osteosarcoma). Collection was performed according to institutional guidelines established by the ethical committees at Kantonsklinik and Kinderklinik in Basel, Switzerland. All patients were treatment-naive at the time of bone marrow collection.

Bone marrow cells were isolated by Ficoll–Hypaque (1.077 g/ml, Pharmacia, Munich, Germany) density gradient centrifugation. Two aliquots of mononuclear cells were stained with mAb’s (Pharmingen, Heidelberg, Germany unless indicated) against: CD19-APC, CD34-FITC, and VpreB-PE (Clone SL688) (Sanz and de la Hera 1996) and CD19-APC, kappa/lambda light chains-FITC, and VpreB-PE. Cellular populations were separated on a MoFlo high performance flow cytometer (Cytometry Inc., Forth
Collins, CO). Cells were sorted directly into Trizol (Heidelberg, Germany) RNA isolation reagent at a maximum of 150,000 cells per 500 μl Trizol, and RNA was extracted according to the manufacturer’s recommendations with 5 μg of Escherichia coli tRNA as coprecipitant. Figure 1A shows a representative example of the sorted cell populations. All cell populations were isolated 5 times from different individuals, so that 5 biological replicates were available.

RNA Amplification and Hybridization to Oligonucleotide Probe Arrays

In vitro transcription-based RNA amplification was performed as described earlier (Hoffmann et al. 2002). In total, 20 μg of labeled cRNA were fragmented and hybridized to the Affymetrix GeneChip HG-U95A arrays, interrogating a total of 12,625 sequences including control genes. GeneChip arrays were washed, stained, and scanned according to the manufacturer’s recommendations, and raw fluorescence intensity images were generated with Affymetrix MAS5 software.

Statistical Analysis of Microarray Data

For quantitative analysis of gene expression levels, raw fluorescence intensity files were first normalized according to the method of Li and Wong (2001a, 2001b) to correct for variations in fluorescence intensities. Next, model-based expression indices were calculated according to the Perfect Match/Mismatch-difference model as described (Li and Wong 2001a, 2001b). Differentially expressed genes were identified by the permutation-based method of Tusher et al. (2001). Briefly, to control for multiple testing, a false-discovery rate (FDR) (Benjamini and Hochberg 1995) was calculated as the percentage of genes falsely detected as differentially expressed among all genes detected as differentially expressed. The q value is the lowest FDR at which a gene is called differentially expressed (Storey and Tibshirani 2003). Gene expression data sets of purified mouse B- and T-cell precursors have been described (Hoffmann et al. 2002, 2003).

When filtering for a common FDR threshold of 20%, the type II error was estimated as follows: In 1,797 human probe sets detected at an FDR threshold of 20%, 1,407 are expected to be truly differentially expressed (80% of 1,797). The entire data set of human mouse homologous probe sets contains 5,650 items with a maximum FDR of 72.19%. We thus expect 1,571 of these probe sets to be truly differentially expressed (100–72.19% = 27.81% of 5,650 probe sets). When filtering for a common FDR threshold of 20%, we thus missed 164 differentially expressed probe sets, corresponding to a type II error of 4.2% (164 missed probe sets/5,650 probe sets total – 1,797 reported probe sets). A type II error analysis for the mouse data set was done accordingly.

Clustering, Functional Annotation, and Identification of Homologous Genes

Hierarchical clustering was performed according to the methods of Eisen et al. (1998) using uncentered Pearson’s correlation as the similarity metric and average linkage clustering on z-score transformed gene expression values. Functional annotations were generated from Gene Ontology (GO) (Ashburner et al. 2000). Statistics for enrichment of GO categories in groups of coexpressed genes were calculated according to a hypergeometric distribution analysis after removal of redundant probe sets by mapping to LocusLink ID’s. Probe sets specific for orthologous genes between mouse and human were identified from the NetAffx database (http://www.affymetrix.com/analysis/index.affx), which is based on reciprocal Blast searches of clustered expressed sequence tag (EST) sequences. Briefly, nucleotide sequences from all UniGene clusters of human and mouse are compared by Blast, and those sequence pairs that share the greatest degree of nucleotide sequence similarity are identified. When sequences from 2 UniGene clusters are reciprocal best matches, the UniGene clusters corresponding to the pair of sequences are considered to represent an ortholog pair. These data are then mapped by Affymetrix on their probe set identifiers, yielding a list of probe sets targeting putative orthologs between mouse and man (see http://www.ncbi.nlm.nih.gov/Web/Newsltr/Spring00/homologene.html). Mappings generated by Ensembl by reciprocal Blast alignments of genomic sequences yielded similar results (see http://www.ensembl.org/info/software/compara/index.html).

Comparisons of Gene Expression Patterns across Species and Developmental Pathways: Identification of Shared Genes

The comparison of gene expression patterns between human and mouse B-cell precursors, or between mouse B- and T-cell precursors, was based on the algorithm described by Lottaz et al. (2006). First, for every developmental stage, a list was generated ranking the genes for the probability of being upregulated compared with all other stages of the same differentiation pathway according to a regularized T statistic. To compare the similarities of 2 lists, the number of genes occurring in both lists above a given rank was determined for every rank. A similarity score was calculated as a weighted sum of these overlaps over all ranks, with the weight decreasing exponentially as the rank increases according to a decay parameter α: w = e−w/n (w = weight, n = rank). α was fixed to 0.01, allowing us to compare the scores derived from different list comparisons. Finally, a rank cutoff is chosen so that the genes that account for 95% of the similarity score are identified as responsible for the similarity of the ordered lists. See Lottaz et al. (2006) for details of the procedure. Source code and windows binary for the R package are available at http://compdiag.molgen.mpg.de/software/OrderedList.shtml. Empirical P values were obtained by permutation testing, using at least 500 random permutations.

Data Availability

Raw gene expression values have been submitted to the NCBI GEO database (http://www.ncbi.nlm.nih.gov/
of genes with these functions is reduced in large Pre-BII cells, which correspond to genes in that particular functional category. The remaining probe sets not upregulated in a stage-specific way are most often upregulated in 2 or more developmentally adjacent stages. One particular exception is a group of 88 probe sets that are expressed in Pre-BI cells, suppressed in large Pre-BII cells, and reexpressed in small Pre-BII cells. As expected, this group contains genes involved in DNA recombination (rag1, rag2). Moreover, it is significantly enriched for transcription factors (\( P = 5.3 \times 10^{-5} \)) and genes involved in DNA packaging (\( P = 3.1 \times 10^{-3} \)). This likely reflects active DNA recombination and dynamic DNA packaging in these largely non-dividing cells.

Results
Gene Expression Patterns in Human B-Cell Development

To generate large-scale gene expression profiles from individual human B-cell precursor stages, we fluorescence-activated cell sorting purified 4 early B-cell precursor populations from bone marrow samples of young donors who underwent iliac crest aspiration for routine workup of non-hematological malignant disease (fig. 1A). RNA was amplified by 2 successive rounds of cDNA synthesis and in vitro transcription as described (Hoffmann et al. 2002) and subsequently hybridized to Affymetrix HG-U95A arrays interrogating 12,625 transcripts. Differential gene expression was assessed employing a permutation-based test correcting for multiple testing (Tusher et al. 2001). At the minimal FDR obtainable (0.11%), 644 probe sets were detected as differentially expressed (fig. 1B). Nine prominent groups of coexpressed probe sets are indicated on the left side of figure 1B. For each cellular stage, there is a stage-specific set of probe sets (Pre-BI cells, 134 probe sets, pre-BCR-positive Pre-BII cells, 30 probe sets, pre-BCR-negative large Pre-BII cells, 46 probe sets, small Pre-BII cells, and 163 probe sets). Pre-BCR-positive and pre-BCR-negative Pre-BII cells have a very similar gene expression pattern (Pearson’s correlation coefficient 0.88, compared with 0.42 between Pre-BI and small Pre-BII cells), consistent with a limited impact of the pre-B cell receptor on cellular gene expression once the signal to initiate proliferation has been transmitted. Figure 1C shows GO “Biological Process” mappings of genes upregulated in Pre-BI, large Pre-BII (irrespective of pre-BCR expression), and small Pre-BII cells.

Two significant shifts in functions of expressed genes during B-cell differentiation become apparent: Pre-BI cells express genes from a variety of functional categories, including “signal transduction activity” (\( P \) value for enrichment 0.017) and “cell adhesion” (\( P = 0.036 \)). Expression of genes with these functions is reduced in large Pre-BII cells. Instead, genes involved in regulation of cell cycle and with “motor activity” become upregulated (\( P < 10^{-6} \) and \( P = 9.7 \times 10^{-5} \), respectively). This shift in functions of expressed genes is almost completely reverted upon differentiation into small Pre-BII cells. This is consistent with the fact that large Pre-BII cells are the only B lineage cells under study which proliferate. All others are resting or have only a minor fraction in cell cycle. Thus, Pre-BI and small Pre-BII cells are prone to actively interact with the bone marrow microenvironment, whereas large Pre-BII cells have a more cell-autonomous gene expression pattern. Consistently, it has been shown that Pre-BII cell expansion occurs independently from pre-BCR signaling (Rolink et al. 2000).

The remaining probe sets not upregulated in a stage-specific way are most often upregulated in 2 or more developmentally adjacent stages. One particular exception is a group of 88 probe sets that are expressed in Pre-BI cells, suppressed in large Pre-BII cells, and reexpressed in small Pre-BII cells. As expected, this group contains genes involved in DNA recombination (rag1, rag2). Moreover, it is significantly enriched for transcription factors (\( P = 5.3 \times 10^{-5} \)) and genes involved in DNA packaging (\( P = 3.1 \times 10^{-3} \)). This likely reflects active DNA recombination and dynamic DNA packaging in these largely non-dividing cells.

Gene Expression Patterns of Human and Mouse B-Cell Development Are Surprisingly Dissimilar

These analyses reflect many known aspects of B-cell physiology and thus demonstrate that the gene expression patterns obtained are meaningful. To compare gene expression patterns of B-cell development between mice and men, we reevaluated a previously described gene expression data set from mouse B-cell precursors (Hoffmann et al. 2002). To facilitate this comparison, gene expression data from human pre-BCR-positive and pre-BCR-negative large pre-BII cells had to be merged because these 2 cellular populations could not be separated in mice due to the low cell surface expression in vivo of the pre-B cell receptor. Thus, 3 consecutive developmental stages are available for a cross-species comparison of gene expression patterns: Pre-BI, large Pre-II, and small Pre-BII cells. Probe sets targeting orthologous genes were identified using an EST-clustering-based approach as implemented in the LocusLink and NetAffx databases (see Materials and methods).

![Gene expression patterns of human B-cell development. (A) Sorting strategy for isolation of human B-cell precursors. CD19-positive, lymphoid cells (R2 in fig. 1) were stained with mAb’s against CD34 and VpreB to isolate CD19+CD34+VpreB+ Pre-BI cells (R4), and CD19+, CD34+, VpreB+ pre-BCR-positive large Pre-BII cells (R3), respectively. For the isolation of pre-BCR-negative Pre-BII cells, CD19-positive lymphoid cells were stained with mAb’s against κ and λ light chains and VpreB, and κ/λ. LC-VpreB-cells (R5) were sorted into large (R6) and small (R7) pre-BCR-negative Pre-BII cells, respectively. (B) Expression patterns of differentially expressed genes in human B-cell development. Genes were normalized to a mean of 0 and standard deviation of 1 across experimental conditions prior to clustering. Red indicates expression level above, and green indicated expression level below the row-wise mean, respectively. Cellular stages are indicated on top, and clusters of coexpressed genes on the left. Pre-BI, Pre-BII cells; + Pre-BII, pre-BCR-positive large Pre-BII cells; − Pre-BII, pre-BCR-negative large Pre-BII cells; sPre-BII, small Pre-BII cells. (C) Functions of differentially expressed genes in human B-cell development. Genes upregulated in individual cellular stages of human B-cell development were identified from the hierarchical clustering (B), and functions were obtained from GO. Functional categories are color coded, and numbers correspond to genes in that particular functional category.](https://academic.oup.com/mbe/article-abstract/24/12/2610/977679)
Therefore, this small overlap is surprising. When expression patterns of orthologous probe sets which are differentially expressed in both human and mouse B-cell development are compared, we find that a larger fraction of these probe sets has a positive correlation (59% of probe sets with FDR < 20% in both human and mouse B-cell development have a correlation of >0.8) (fig. 2A), and this fraction of positively correlated probe sets increases with more stringent FDR cutoffs (data not shown). However, the overlap between human and mouse probe sets when filtering for a common significance FDR still is surprisingly small: Between 1,797 human and 518 mouse probe sets that reach a FDR of 20%, only 371 orthologous pairs involving 306 human and 323 mouse probe sets could be identified. A type II error analysis estimates the type II error as 4.2% in the human and 0.4% in the mouse data set, indicating that at most 21 human–mouse homologous probe sets may have been missed in our analysis. As the type I error rate is estimated to be below 5% in both data sets, we do not believe that the low correlation between human and mouse gene expression results from a “random subsampling” of genes from an underpowered experiment.

It is clear, however, that the human and mouse data differ in power. Filtering for a common significance threshold in 2 experiments with different power will obviously miss some differentially expressed genes from the list with lower power, and the magnitude of this effect will be dependent on the critical significance threshold used. To circumvent this problem, we developed an algorithm that is independent from fixed significance thresholds; rather, it quantifies similarities of probe set lists ranked for probability of differential expression. Briefly, for every developmental stage of human and mouse B-cell development, a regularized t-score is used to rank orthologous probe sets according to their likelihood of being induced compared with the remaining 2 stages. For every rank, the number of orthologous probe sets occurring in both human and mouse gene lists above that rank was recorded. A similarity score was defined as a weighted sum of overlaps per rank, attributing weights to the top of the list. Finally, probe sets were identified which contribute to the similarity score and are thus likely to be involved in both human and mouse B-cell development.

Figure 2B shows the rank-based overlaps of lists comprised of human and mouse probe sets which are upregulated in pre–BI, large, and small pre–BII cells compared with the remaining 2 cell populations, respectively. This overlap is higher than would be expected by chance (Random, overlap between randomly permuted lists, \( P < 2 \times 10^{-3} \)) but nevertheless surprisingly small: at an arbitrarily chosen threshold of 300 top-ranking probe sets, 28 probe sets overlap between the pre–BI cells of human and mice, 104 probe sets overlap in large cycling pre–BII cells, and 39 probe sets overlap in small pre–BII cell gene lists, compared with 11 overlapping probe sets in a randomly permuted gene list. Thus, only relatively few probe sets are upregulated in comparable stages of mouse and human B-cell differentiation. Similarity scores for lists of probe sets upregulated in human and mouse pre–BI, large pre–BII, and small pre–BII cells are 884, 3,203, and 969, respectively, compared with 238 for randomly permuted lists.
Thus, large pre–BII cells of mice and men have the most similar gene expression pattern, whereas pre–BI and small pre–BII cells are more dissimilar. The similar genes are cell cycle related, indicating a conserved usage of genes involved in cell cycle control between different species.

By using our method for comparison of ordered probe set lists, we identified sets of probe sets targeting orthologous genes, which are induced in the same cellular stage of human and mouse B-cell development. These genes can be considered a “core set” most likely to be responsible for the cellular phenotype in different species. Figure 3 shows the distribution of several major GO categories of 65, 141, and 96 probe set pairs (mapping to 48, 86, and 75 distinct human genes, respectively), which are significantly associated with both human and mouse Pre–BI, large Pre–BII, and small Pre–BII cells. When testing overrepresentation of GO categories for statistical significance, genes shared between human and mouse Pre–BI cells are enriched for GO categories metabolism (P \( < 2 \times 10^{-3} \)) or biosynthesis (P \( < 0.023 \)). By contrast, genes shared between human and mouse large Pre–BII cells are enriched for cell cycle (P \( < 1 \times 10^{-6} \)), cytoskeleton (P \( = 4.5 \times 10^{-4} \)), or DNA replication (P \( < 1 \times 10^{-6} \)) genes, whereas genes shared between human and mouse small Pre–BII cells are enriched for immune response (P \( = 0.03 \)) or signal transduction (P \( = 0.034 \)) genes. Thus, conservation of gene expression patterns during evolution involves different genes for different cell types.

The Relative Impact of Species and Functional Specialization on Gene Expression Patterns of Developing Lymphocytes

In order to provide a framework for interpretation of the comparison between human and mouse B-cell development gene expression patterns, we have performed a similar analysis for corresponding stages between mouse B- and T-cell development. The ordered lists of probe sets upregulated in Pool I (Pre-BI and DN2/DN3), II (large Pre-BII and DN4/DP1), and III (small Pre-BII and DP3) cells (Hoffmann et al. 2003) reach similarity scores of 942, 4,982, and 782, respectively (compared with 151 of randomly permuted probe set lists) (fig. 4A). Thus, much like in human and mouse B-cell development, cells undergoing proliferative expansion after the first wave of rearrangements have the most similar gene expression profiles, whereas the other stages are more dissimilar. We next compared the overlaps of human–mouse B-cell differentiation with those of B–T cell differentiation (fig. 4B–D). For early stages of lymphocyte differentiation (fig. 4B and C), mouse B- and T-cell precursors have more overlaps in ordered gene lists than human and mouse B-cell precursors. For more mature stages (fig. 4D), this trend is reversed; human and mouse B-cell precursors have higher overlaps than mouse B- and T-cell precursors. This indicates that early in hematopoiesis, cells on their way to different lineages of differentiation are still close to each other. Thus, prior to acquisition of cell-type–specific functions, gene expression patterns are
similar even for different cellular differentiation pathways within a given species. As the cells mature and functional specialization occurs, however, gene expression patterns become more similar across species for a given differentiation type and more different across related differentiation pathways (fig. 4E).

Discussion

The present study describes gene expression profiles of 4 consecutive stages of human B-cell development. To obtain sufficient numbers of B-cell precursors, bone marrow specimens of very young donors have to be used.
because B-cell generation decreases in older individuals (Caldwell et al. 1991; Ghia et al. 1996). The bone marrow specimens for this study were obtained from children on whom iliac crest aspirations were performed during the initial workup of newly diagnosed malignant disease. We have no reason to suspect that the B-cell lineage populations in these patients were influenced by the disease. None of the patients had neoplasia of the hematopoietic system or has been treated with chemotherapy or irradiation prior to specimen collection. Moreover, the FACS-profiles of B-cell lineage precursors were comparable between the different patients, indicating that the different diseases did not affect B lymphopoiesis.

We could demonstrate that only a relatively small proportion of identified orthologous genes are upregulated in the same B-cell developmental stage of both mice and man. This is in line with several recent studies investigating gene expression patterns during evolution. Gene expression patterns in brains, livers, and peripheral blood from primates and rodents are substantially different even between closely related mammalian species (Enard et al. 2002), with the extent of conservation depending on tissue type (Khaitovich, Hellmann, et al. 2005). Correlation of expression changes in *Caenorhabditis elegans* and *Drosophila melanogaster* during aging are low, albeit significantly higher than would be expected by chance (McCarroll et al. 2004), and the chimpanzee brain undergoes different gene expression changes during aging than the human brain (Fraser et al. 2005). Such expression differences between species have been suggested to be of little functional consequence because they are approximately proportional to the time since species divergence (Khaitovich et al. 2004; Yanai et al. 2004). This has been termed “neutral model of transcriptome evolution.” However, this view has recently been challenged as this accumulation over time could not be demonstrated when using gene expression data from common ancestors as outgroups (Gilad et al. 2006).

Our data, however, argue in favor of a selectively neutral evolution of expression patterns for the majority of genes which are differentially expressed. This interpretation is based on the assumption that mouse and human B-cell development are functionally similar developmental pathways. Alternatively, one could argue that the gene expression differences reflect functional differences between human and mouse B-cell development. We do not, however, believe that this is the case. Mouse and human B-cell precursors form similarly sized compartments and share the biochemistry of V(D)J recombination, which is the key molecular process involved in generation of B cells. Moreover, deficiency in components of the pre-B-cell receptor results in similar phenotypes in man and mouse (Ghia et al. 1998). Finally, the function of B cells, namely production of antibodies upon antigen-specific stimulation of the B-cell receptor, is the same in man and mouse. We thus consider it unlikely that human and mouse B-cell differentiation is functionally sufficiently dissimilar to explain the extent of gene expression differences observed here. In fact, we think that this model of B-cell differentiation is particularly well suited to investigate transcript evolution because the function of a B cell is much better preserved through evolution than, for example, the function of different brain regions.

It should be noted that there may be an alternative interpretation of our data. There has been strong stabilizing selection for the phenotype of B-cell differentiation as this trait is conserved from mouse to man. If nonneutral differences in gene expression occur through evolution (e.g., by mutation of transcription factor coding or recognition sequences), one could expect a second, compensatory change in gene expression to occur in order to stabilize the cellular function. Thus, one could envision a scenario where nonneutral and compensatory expression differences accumulate, resulting in cell populations with similar functions and rather dissimilar, and thus seemingly functionally neutral, gene expression patterns. Such “compensatory evolution” has been described for sequences of, for example, *Drosophila* enhancers, where “sequence differences between species have functional consequences [...] but they are masked by other coevolved differences” (Ludwig et al. 2000). It should be noted that accumulation of nonneutral and compensatory expression changes is expected to occur approximately linearly over time and is thus consistent with the findings from which the neutral theory originally evolved (Khaitovich et al. 2004; Khaitovich, Paabo, et al. 2005).

The neutral model of transcriptome evolution (may it be amended by compensatory aspects or not) suggests that a small set of functionally relevant genes with conserved sequence and expression pattern during evolution exists (Khaitovich, Paabo, et al. 2005). These expression patterns may have been positively selected in an ancestor of mice and humans and have since been negatively selected, that is, conserved. We find that expression patterns of a small number of genes (often related to cell cycle) appear to be conserved since species diversification of mouse and man. We suggest that these genes may be negatively selected and highly relevant for the cellular phenotype.

Our comparisons of intra- and interspecies differences in gene expression patterns indicate a graded impact of different factors during lymphopoiesis. In early stages of B and T-cell development, gene expression patterns are more similar within than across species. Thus, in the absence of functional specialization, mouse B- and T-cell precursors follow a similar transcriptional program, and species differences exceed lineage differences (this may be termed ontogenic impact). As functional specialization occurs, this trend is reversed, and the B cells of mice and humans become more similar. Thus, the lineage differences now exceed the species differences in gene expression (this may be termed functional impact).

From our comparative analyses of gene expression patterns in human and mouse lymphocyte development, we conclude the following:

1. Expression differences between human and mouse B-cell precursors suggest an amended neutral model of transcriptome evolution, where expression differences accumulate so that the cellular function is maintained. This may be achieved by functional neutrality or by coevolution of compensatory expression differences.

2. A small set of genes with similar expression patterns in mice and man may be negatively selected and highly relevant for shaping the cell’s phenotype.
3. The gene expression pattern of developing lymphocytes is shaped by ontogenic factors in early stages and by cell-type–specific functions in more mature stages.

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Literature Cited


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