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Immune System Promiscuity in Human and Non-human Primate Evolution

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Abstract

Many genes that respond to infection have functions outside of immunity and have been found to be under natural selection. Pathogens may, therefore, incidentally alter non-immune physiology through engagement with immune system genes. This raises a logical question of how genetically promiscuous is the immune system; here defined as how heavily cross-referenced is the immune system into other physiological systems? Here, we examine immune gene promiscuity across physiological systems in primates by examining the baseline (unperturbed) expression of key tissue and cell types for expression differences, and primate genomes for signatures of selection. We found “immune” gene expression to be cross-referenced extensively in other physiological systems in primates. When immune and non-immune tissues diverge in expression, the differentially expressed genes at baseline are enriched for cell biological activities not immediately identifiable as immune function-based. Individual comparisons of immune and non-immune tissues in primates revealed low divergence in gene expression between tissues, with the exception of whole blood. Immune gene promiscuity increases over evolutionary time, with hominoids exhibiting the most cross-referencing of such genes among primates. We also see positive selection in the coding regions of differentially expressed genes between tissues functionally associated with immunity. This suggests that with increasing promiscuity, divergent gene expression between the immune system and other physiological systems tends to be adaptive and enriched for immune functions in hominoids.

The human immune system is a complex, multi-tiered system of cellular, tissue and protein components that comprises the primary defense of our species against invading micro-organisms. It is an ancient, energetically expensive biological system that is fundamental to the survival of living things. Given its importance, it should not be surprising that the immune system is under tremendous selective pressure and that the genes involved can rapidly diversify (Barreiro et al. 2008; Cortez et al. 2017; Ferrer-Admetlla et al. 2008; Kosiol et al. 2008; Novembre and Han 2012; Pickrell et al. 2009; Wang et al. 2017). Pathogen-mediated selection tends to be viewed as the primary force influencing human immune system evolution (Ferrer-Admetlla et al. 2008; Fumagalli et al. 2011). Eukaryotic life, after all, was born into an environment where prokaryotes, viruses and archaea were already competing for resources via infection (Casanova 2015). Moreover, humans have evolved in a diverse microbial landscape, under profound, routine assault by pathogens and have carried a heavy disease burden for most our of existence (Fumagalli et al. 2009; Harper and Armelagos 2010; Harper and Armelagos 2013; Karlsson et al. 2014). Prior interactions with microorganisms appear to be reflected in our genomes. Genes with clear immune functions tend to comprise the largest functional categories of positively selected genes in human populations and across mammalian species (Kosiol et al. 2008; Vallender and Lahn 2004). While pathogen-mediated selection is no doubt an extremely important factor driving the evolution of the species, this framework of immune system evolution prioritizes microbial conflict as *the* factor shaping immunity, and obscures the importance and impact of the system being highly cross-referenced physiologically on genomic evolution (Brinkworth 2017).

The immune system is very physiologically promiscuous, stretching into every tissue, lending and co-opting genes to and from the developmental and cellular programs of “non-immune” systems. Indeed, most genes that fulfill immune functions are pleiotropic and also

engaged in activities traditionally considered to be “non-immune” (Gosselin et al. 2005; Hattermann et al. 2008; Johnson et al. 2009; Mori et al. 2016; Piccinin et al. 2010; Sferruzzi-Perri et al. 2009; Sheridan and Murphy 2013). The ancient origins of immunity and the increasing complexity of interactions between microorganisms and hosts over evolutionary time likely partially explain the multifunctional and cross-system infiltrative nature of immune system components. The “borrowing” and “lending” of immune system building blocks has led to multifunctionality in many of the components of the system, with associated genes appearing to have evolved in parallel with other systems, functions, and processes (Adler and Rogers 2005; Ambrosi et al. 2014; Kitaya et al. 2007; Reaux-Le Goazigo et al. 2013; Takizawa et al. 2008). System genetics approaches over the last decade have well established that most phenotypes, including characteristics of immunity, are controlled by pleiotropic networks of many genes (reviewed in Boyle et al. 2017; Wagner and Zhang 2011) (most recently referred to as “the omnigenic model of complex traits” Boyle et al. 2017). While the evolution of single pleiotropic genes may sometimes be constrained due to their role in multiple phenotypes (Wagner and Zhang 2011), selection on phenotypes expressed via genes networks means that adaptive shifts in target genes are accompanied by small functional shifts in many other networked genes to compensate for changes at one physiological interface (Pavlicev and Wagner 2012). An important aspect of human immune gene pleiotropy is that many of the genes in question are involved in the development of tissues that manifest features considered to be particularly human and hominoid [e.g. forebrain development and brain volume (Kee et al. 2003; Luo et al. 2012; Tsai et al. 2002), cognitive function (Ben Menachem-Zidon et al. 2008; Bliss and Collingridge 1993; Meola et al. 2013) and embryogenesis and placental invasion (Chatterjee et al. 2013; Das et al. 2002; Kitaya et al. 2007; Piccinni et al. 1998; Sferruzzi-Perri et al. 2009; Takahashi et al.

2006). In this context, factors driving the adaptive evolution of immunity may be contributing to the divergence and diversification of other traits and vice versa. More specifically, it should be expected that evolutionary interactions with pathogens could mediate changes in heavily immune cross-referenced physiology considered important to the human evolutionary story.

In this review, we illustrate the cross-referencing of the immune system in other physiological systems, and, broadly, the degree to which immune tissues transcriptionally diverge from non-immune tissues using evolutionary immunology and genomic approaches to analyze previously published data sets (Clark et al. 2015; Derrien et al. 2012; Lin et al. 2014; Linsley et al. 2014; Peng et al. 2015). We contrast genome-wide gene expression of primate immune and non-immune tissues by species and tissue type, as well as examine differentially expressed (DE) genes to identify divergent functions and expression specificity across species. Lastly, we assess DE genes for signatures of positive selection. We show that “immune” gene expression is extensively cross-referenced in other physiological systems in primates. These genes show high overlap in expression between immune and non-immune tissue types and are enriched for a mix of cell biological activities both immune and non-immune in nature. Our analysis here indicates that this cross-referencing increases over evolutionary time in primates, with positively selected divergent gene expression in hominoids enriched for immune function.

Multiple Human “Non-Immune” Tissues Have Been “Immune” Tissues in the Past The highly networked and promiscuous nature of the immune system is, at once, a strong indication of the importance of host defense in species survival and an outcome of a very ancient system expanding through extensive genomic change over time. With novel species-specific changes in expanded and restricted immune gene families and differences in immune tissue development

and immune cell content the human immune system is a relatively new configuration of mammalian immunity (Boehm et al. 2012a; Brinkworth and Thorn 2013). Over the course of vertebrate evolution, for example, organs and tissues not considered to be involved in human immunity have been key generators of immune cells and components. B cell development in mammals, for example, is completed almost entirely in the bone marrow with minor activity occurring in the spleen. In fish and amphibians, B cells extensively develop in other locations such as the kidneys (cartilagenous and bony fish), spleen (cartilagenous fish, amphibians) and liver (amphibians)(Boehm et al. 2012a; Boehm et al. 2012b; Du Pasquier et al. 2000; Greenhalgh et al. 1993; Pickel et al. 1993). Secondary lymphoid tissues/organs are usually differentiated structures of organs that have entirely different primary functions but are at the interface of the host's internal and external environments. GALT extensively develops in the gut tube, the central component of the digestive system. The spleen is primarily a blood filter, though it is also a site for lymphocyte development and the action of filtering has been cited as having an immune-like effect as a possible reason for malaria resistance in sickle-cell anemia carriers (Kwiatkowski 2005). Similarly, the genes the immune system have undergone co-option over the course of immune system evolution. Some of the earliest genes identified as initiating immune responses against invading pathogens, Toll-like receptors, are engaged in determining the ventral-dorsal axis (Ambrosi et al. 2014; Brennan et al. 2017) in invertebrates. Chemokine and cytokines genes, which are key to first line of defense immune responses, including cell trafficking, inflammation and immune cell differentiation, are extensively cross-referenced in forebrain/brain development and neurogenesis [(e.g. CXCL12, CXCR4, CXCL16, CXCR6, IL-3)(Hattermann et al. 2008; Klein et al. 2001; Luo et al. 2012; Reaux-Le Goazigo et al. 2013; Reiss et al. 2002)]. A host of chemokines engaged in antimicrobial activities are also key to

normal neurological function [(CXCL12, CXCR4, CXCL8/IL8 , CCL2, CCR2, CXCL31)(Adler and Rogers 2005; Giovannelli et al. 1998; Gosselin et al. 2005; Piccinin et al. 2010)] and cognitive memory [CX3CL1, CX3CR1, reviewed in (Sheridan and Murphy 2013)]. Similarly, cytokines are very actively engaged reproduction, including embryo implantation [reviewed in (Mori et al. 2016)], trophoblast differentiation [CSF2, (Sferruzzi-Perri et al. 2009)], placental invasion and development (IRF1, STAT1)(Johnson et al. 2009; Kitaya et al. 2007) , decidua/maternal blood pressure regulation [IL-4, IL-40, CSF1, LIF (Chatterjee et al. 2013; Piccinni et al. 1998; Piccinni et al. 2001)] and platelet architecture and embryonic hematopoiesis [cytokine regulator SH2B3, IL-7 (Dravid et al. 2011; Takizawa et al. 2008). This cross-referencing of immune genes with “non-immune” tissues means that *evolutionary interactions with pathogens may be altering non-immune phenotypes through direct interactions with genes in the responsible gene network.*

Primates Respond to Bacteria with Genes Engaged in Non-Immune Activities From an evolutionary standpoint the net effect of immune promiscuity may be that pathogens interacting with the immune system exert direct selective pressure on genes responsible for both immune and non-immune phenotypes. To illustrate that possibility, we assessed the top 10 upregulated genes that responded in human, chimp and rhesus macaque monocytes stimulated with lipopolysaccharide derived from *Escherica coli* by Barreiro et al (2010), against the Genecard(www.genecard.org) and On Medelian Inheritance in Man (<https://omim.org/>) databases, as well as an extensive literature search for known functions (Institute 2018; Stelzer et al. 2016). Here, the “top ten upregulated” is an amalgam of the 10 genes most strongly upregulated as measured by the differential expression across all three species. This simple

examination revealed the majority of genes (70%) strongly upregulated in response to challenge by Gram-negative bacteria are involved in two physiological systems that significantly inform reproductive success, the reproductive tract and the central nervous system. These findings broadly agree our prior examination of multiple human cell whole genome responses to bacterial (*Mycobacterium tuberculosis*), viral (influenza A) and parasitic (*Trypanosoma cruzi*) pathogens that examined the top five upregulated responding genes shared across cell infections and found they were highly multifunctional and played important roles in other physiological systems such as digestive and reproductive systems (Brinkworth 2017). Similarly, we have previously found that “immune” genes both associated with a chronic inflammatory and autoimmune diseases and signatures of positive selection in human are also involved in important “non-immune” related processes such as uterine remodeling, decidua regulation, respiration, brain development, and sensory motor gating (Brinkworth and Barreiro 2014). In the current analysis, at least five of the ten genes responding to LPS appear to be under mild to strong purifying selection ($dn/ds = 0.01-0.85$, marked with * in Table 1) in primates, and are cross-referenced in central nervous system function (Table 1). In combination with previous results, the current examination suggests that, despite pleiotropy, genes responding to pathogen stimulus and involved in non-immune functions are not always evolutionarily constrained by their multiple roles. The significant upregulation of genes involved in non-immune activities in response to pathogen stimulus that are also under stronger purifying selection supports the possibility that pathogen-immune system evolutionary interactions can affect the function of non-immune systems.

A Large Proportion of Genes Expressed in Non-Immune Tissues at Physiological Baseline

Have a Role in Immunity: Genetic Promiscuity In combination, the multifunctional nature

of genes responding to immune challenge and evidence that these responding genes are under natural selection supports the hypothesis that pathogens may incidentally alter non-immune physiology through engagement with immune system genes. This raises a logical question of how *genetically promiscuous is the immune system in primates*; here defined as how heavily cross-referenced into other physiological systems is the immune system in primates? We compared the whole genome gene expression (RNA-Seq) of 8 immunological and non-immunological tissue types in 5 primate species at physiological baseline (i.e. tissues not known to be infected, injured or otherwise unperturbed, hereafter referred to as at “baseline”) using transcriptomes made publicly available by the Non-Human Primate Reference Transcriptome Resource (NHPRTR), or published in peer-reviewed journals and archived the Genome Expression Omnibus (Clark et al. 2015; Derrien et al. 2012; Lin et al. 2014; Linsley et al. 2014; Peng et al. 2015). Primates included in the study were human (*Homo sapiens*), chimp (*Pan troglodytes*), rhesus macaque (*Macaca mulatta*), common marmoset (*Callithrix jacchus*), and the mouse lemur (*Microcebus murinus*). Tissues were categorized as “immune” vs “non-immune” based on current known function in cellular immunity. Under this scheme colon, lymph node, spleen, thymus and whole blood represent primary and secondary lymphoid sites and were categorized as “immune”, and kidney, liver and brain were categorized as “non-immune” (Table S1). As the evolutionary distance between species grows, the number of genes expressing species-level programs increases (Lin et al. 2014) (Pishesha et al. 2014). Several previous studies that have used transcriptomic data from multiple organs and multiple-species (e.g. organ differences among amniotes) to look at relative rates of change in organs over time. Those studies have either directionally scaled their data or limited the dataset to tissue-specific gene expression (Barbosa-Morais et al. 2012; Brawand et al. 2011; Breschi et al. 2016; Merkin et al.

2012). Here we are doing something different. Since we seek to highlight genes that are expressed across physiological systems in *Primates*, we have not directionally scaled the transcriptomic data as doing so would mask this information. A principal components analysis on Spearman's rank correlations of normalized expression of 8869 genetic orthologs for all tissues (full dataset), as well as a reduced matrix of only tissues available for all 5 species reveals that 75.9-82.1% of the variation expressed (PC1) is explained by membership in the family hominidae (Figures S1 and S2). By contrast, very little gene expression variation (2.6%) (PC4) in the full dataset is explained by a tissue belonging to the "Immune" or "Non-Immune" categories, a finding consistent with promiscuous gene expression (Figure 1). This ordination on the PCA plot is due to the choice of scaling mentioned above.

To identify how many genes broadly categorized as "immune genes" are expressed in tissues considered to be non-immune, we used the genes identified by Gene Ontology consortium as "immune system process" (3553 genes). We then compared these genes in humans against 3 "non-immune" tissues expressed in the 5 primate species. In the grouped non-immune tissues, 1352 of 8869 (15.2%) orthologous "immune system process" genes were consistently expressed above a counts per million normalized value of ≥ 1 (Table S2). *Homo-Pan and Homo-Macaca* comparison of coding regions, revealed 44 of these cross-referenced genes with a dN/dS ratio of over 1.2 suggesting they may be under positive selection in humans. A more refined comparison seeking possible cross-referencing of genes that are engaged in immune responses (GO: "activation of immune response") in humans (826 genes) found that 301 genetic orthologs (3.6% orthologs, and 36.4% of the GO category) are consistently expressed (cpm ≥ 1) in these non-immune tissues as a group. Twelve of these genes appear to be under positive selection in humans (dN/dS > 1.2), and not in the other comparisons. This data lends support to

the notion that a significant number of genes active in response to infection play a role in the function and maintenance of “non-immune” tissues, and that evolutionary change in genes categorized as “immune” may affect function in non-immune tissues.

Divergent Gene Expression between Immune and Non-Immune Tissue Is Associated with

Basic Cell Function, Not Immunity That the entire “immune system process” gene list is not cross-referenced in genes expressed at baseline in primate non-immune tissues is not surprising. Logically, immune and non-immune tissues must differ in gene expression as well. Given that pathogens play a strong role in the evolution of the immune system, it would be reasonable to expect that strong differences in baseline gene expression between immune and non-immune tissues occur in genes with known immune functions. When non-immune tissue gene expression is contrasted against gene expression in immune tissues across all 5 species, 16.3% (1448) genes are found to be significantly differentially expressed (DE) (20% fold change and a Benjamini-Hochberg false discovery rate <0.1) (Table S3). However, when these genes are examined for GO Biological Process category enrichment (overrepresentation analysis, GOrilla/Revigo) they appear to be mainly engaged in activities other than immune function (allowed similarity of 0.7, SimRel algorithm, $fdr < 0.001$) (Eden et al. 2009; Supek et al. 2011). The most strongly enriched GO Biological Process categories were found to be semantically similar to *Ion transmembrane transport* (q-value = 8.1×10^{-9}) a biological process of membrane transport that is thought to have diversified significantly by cell type and transported substrate (Attwood et al. 2017), and three categories that could be lumped into cellular signaling, including *Regulation of biological quality* (q-value = 5.13×10^{-3}), *Single organism signaling* (q-value = 5.37×10^{-4}) and *Protein activation cascade* (q-value = 2.6×10^{-5}) (Figure 2)(Table S4: Gorilla/REVIGO results).

This is true also of the 838 (9.4%) genes that are upregulated in immune tissues vs non-immune tissues,) (20% fold change, $fdr < 0.1$), where the most strongly enriched GO Biological Process categories were found to be semantically similar to *Response to metal ion* , and include organic acid metabolic process, carboxylic acid metabolic process, oxoacid metabolic process ($q\text{-values} = 8.15^{21}, 1.15^{20}, 4.70^{20}$) (Table S4: Gorilla/REVIGO results). While cell signaling often includes cytokines, which complete antimicrobial activities directly and through cell communication, few other DE genes in these categories have known antimicrobial functions (Selmaj et al. 1991). It appears that gene expression between “immune” and “non-immune” tissues has primarily diverged in functions such as membrane transport and cell signaling that may impact immune activities, but are not restricted to nor necessarily immediately identifiable as immune function-based.

Non-Immune Tissues Exhibit Low Divergent Gene Expression from Almost All Other Tissues

A byproduct of the cross-referencing of genes between the immune system and other physiological systems should be homogeneity in differently expressed genes between immune tissues and non-immune tissues. A more granular analysis of tissue type gene expression reveals a gene transcription pattern supporting this notion. When the gene expression values of each tissue are contrasted pairwise across species (fold change 20%, $fdr < 0.1$) individual non-immune tissues were found to minimally diverge in expression from immune and non-immune tissues (Figure 3)(Table S3). The exceptions to this pattern included mainly NI tissue contrasts against whole blood, an immune compartment that maintains a very high concentration and diversity of immune cell types. Those contrasts produced a low-moderate number of DE genes (kidney – whole blood: 559; brain – whole blood: 964; Liver – whole blood: 639 genes). Low-moderate

divergence was also noted for whole blood contrasted against colon (492 genes) and the contrast of liver against the brain (771 genes). When the DE genes of the whole blood contrasts were examined for gene enrichment across a broad range of function (GO Biological Process), phenotype/disease (Jensen Disease; dbGaP) and cell (Human Gene Atlas) ontogenies via the program ENRICHR a distinct pattern of enrichment for a mix of immune and non-immune traits emerges (Kuleshov et al. 2016). All non-immune tissue - whole blood contrasts produced DE genes enriched for the phenotypes of “body height” (mainly growth factors used throughout life) and potentially anti-microbial cholesterol type, along with the immune biological process of neutrophil degranulation, and the disease trait carcinoma (Table S5)(Chen et al. 2013; Kuleshov et al. 2016). Contrasts with the brain or kidney and thymus produced strong enrichments for T-cell activity, highlighting the mammalian thymus specialization in T-cell development. A mixed pattern was noted for the brain-liver contrast, not an immune vs non-immune tissue contrast, where DE genes tend to be enriched for categories associated with immunity (carcinoma) as well as more general cell function such as synaptogenesis and signal peptide processing. Interestingly, when the remaining 25 tissue contrasts that exhibited DE genes were examined for DE gene enrichment, 64% (16/25) were enriched for carcinomas, suggesting that the regulation of genes associated with cancer has evolved differently in different tissues, and across human and non-human primates, both notions which have been explored experimentally (Babbitt et al. 2017; Pizzollo et al. 2018). With the exception of the colon-whole blood contrast, immune tissues exhibited very minor if any divergent gene expressed compared to other immune tissues.

Gene Expression across Immune and Non-Immune Tissues Becomes More Promiscuous over Evolutionary Time, and Is Most Promiscuous in the Hominoid Lineage Though immune

system genes are promiscuous in their expression across tissues in primates, there is ample evidence that primate immune systems have diverged in genomic responses to infection and cell physiology (Advani et al. 2016; Barreiro et al. 2010; Brawand et al. 2011; Brinkworth et al. 2012; Pizzollo et al. 2018). This suggests that along with the multifunctional nature of immune system genes, there is species-specificity in immunogenomic activity in primates. Species-specificity in immune gene activity tends to be described as either novel elements (Chuong et al. 2016; Gombart et al. 2009), and highly divergent gene code or copy number (Kehrer-Sawatzki and Cooper 2008; Perry et al. 2008; Romero et al. 2017). It is also, on occasion, described as genomic responses to immune challenge that are significantly up or down regulated over or between particular false discovery rates in one species, but not over or between particular false discovery rates of a second or multiple species in the study in question (Barreiro et al. 2010; Tian et al. 2018; Valenzuela-Munoz et al. 2017). From a functional standpoint, evolving non-immune phenotypes involve multifunctional genes also associated with immunity. Species may evolve unique immune responses through subtle, networked alterations in the promiscuity of genes associated with both the immune system and other physiological systems. These changes can't be detected using the first two methods mentioned above, and their exploration would benefit from analytical tools that can complement the use of multiple p-value thresholds as a means of assessment. One method for addressing the possibility of species-specific gene expression promiscuity is to compare the specificity of gene expression across species representing major clades of primates using a scoring system that has been used to examine tissue specificity of gene expression across tissues and species, that controls for the magnitude of expression and so makes head-to-head comparisons possible (Babbitt et al. 2017; Haygood et al. 2010; Haygood et al. 2007). For each gene and tissue, we computed a specificity score between 0 and 1 representing

the specificity (Haygood et al. 2007; Kosiol et al. 2008) of the gene's expression to the tissue (I vs NI), which can also be expanded across species pairs (Babbitt et al. 2017). The specificity score is high if the expression is very specific to the contrast across species, and is low even for the gene's tissue of maximal expression if the gene nearly as highly expressed in other tissues.

Specificity of gene expression changes the distribution of genes that are specific to either I or NI tissues over evolutionary time. In this analysis, the input values are the log Fold Change in Immune vs Non-Immune tissues. There is one distribution for each species, allowing for the species to species contrast. The distributions represent specificity values (where 0 shows even expression in all tissues to 1 where gene expression is specific to a given set of tissues). There is less contrast-specific expression (I vs NI) in the human-chimpanzee comparison, as compared to human and *Macaca mulatta*, *Callithrix jacchus*, and *Microcebus murinus* (Figure 4). The number of contrast-specific genes increases with evolutionary time. This suggests there is more promiscuous gene expression across NI and I leading to the hominoid lineage, as compared to the other primate species.

Conversely, the genes that are more evenly expressed across all the tissues can be examined. With these genes (specificity < 0.2), GO Biological Process enrichments in housekeeping categories such as "protein phosphorylation", "spliceosomal complex assembly", and "histone phosphorylation" are apparent. Seeing, as might be expected, categories essential to cellular function, and not necessary to specific tissue biological processes.

There Are Correlations with Positively Selected Genes and Differences in Expression The high promiscuity of gene expression across immune and non-immune tissue leads to low divergence in gene expression between such tissues in primates. That such gene sharing is

common and possibly adaptive, suggests that for gene expression to significantly depart from sharing, such singular gene expression patterns are beneficial. To intersect these patterns with signatures of selection, the overlap between DE and signatures of positive selection in immune vs non-immune tissues was examined. Signatures of positive selection were defined by dN/dS ratios across species comparisons taken from Ensembl (Zerbino et al. 2018). In both sets of tissues we see a weakly positive, but significant, correlation in both sets of tissues when looking at human-chimpanzee dN/dS ratios. For the immune tissues there is a weak but significant correlation (permutations p-value < 0.0001, rho=0.0967, n=147, 20000 simulations); a similar pattern is seen in non-immune tissues (permutation p-value = 3e-04, rho=0.0996, n=84, 20000 simulations). Enrichment analyses on protein-coding regions showing evidence (dn/ds > 1, n= 430 orthologs) of accelerated change show enrichments in diffuse immune and transport categories, such as “cytokine-cytokine receptor interaction”. In contrast, enrichments using the human-macaque comparison (dn/ds > 1, n= 81 orthologs) show a stronger enrichment for immune response categories and their roles in human disease (Table S6). Suggesting that these differences are more readily apparent at slightly longer evolutionary distances, and that those rates of change may inform how global adaptive immune changes have impacts over evolutionary timescales.

Discussion

The degree to which multiple traits are affected by pleiotropy has come into focus recently due to more precise measurements of both genotypic and phenotypic changes (Cannell et al. 2015; De et al. 2014; Li et al. 2017). It has become progressively apparent that pleiotropy is modular and somewhat constrained, allowing for evolvability (reviewed in Wagner and Zhang, 2011). The

extent of pleiotropic interactions between tissue functions, such as immune/non-immune compartments, has not been explored in the study human evolutionary biology. In this review of immune promiscuity, we offer an analysis to explore how tightly associated these different functions are across tissues and evolutionary time.

The nature of the underlying gene networks, however, are still unclear. There can be tissue-specific effects for genes, even genes that are widely expressed (Bossi and Lehner 2009). The degree of pleiotropy for most genes has been estimated to rather small, (reviewed in Wagner and Zhang 2011), however such assessments suffer from a lack of information about gene expression over the course of an organism's development. There is no ontological series of gene expression available for all of these primate species and tissues. Acquisition of such data could enhance our understanding of the interplay of these genes over life-histories, as many of the cross-referenced genes might be turned on and off during development. A better relief of how cross-referenced genes are transcribed and silenced over the life course can provide important information on evolutionary trade offs between the immune and other physiological systems. Importantly, such data can reveal how experiences in early life that impact immune gene expression can alter physiological function of other body systems and health later in life.

As we have shown here, the immune system is very promiscuous, and in primates, this increases over the evolution of the order. We also see evidence of accelerated changes between I and NI gene expression in the hominoid lineage. Immune and non-immune tissues show low-moderate divergence in expression, yet, when they do, the functional enrichment of those genes is for a mix of immune and non-immune characteristics – suggesting that this interplay between systems is driving a host of phenotypes to diverge. When they do diverge there is strong enrichment for disease susceptibility genes underlying a common human disease, cancer.

As for possible adaptive consequences, we also see that the DE genes between immune and non-immune tissues under positive selection in hominoids appear to be enriched mainly for immune function. This suggests that immune functions are shaping other phenotypes. An exciting prospect of increasingly accessible gene editing technology is that very controlled assessments of the impact of immune divergence on non-immune phenotypes and the many specific mutations that may be involved can be readily initiated in animal or cell models.

Taken together, the net effect of immune promiscuity may be that pathogens interacting with the immune system exert direct selective pressure on genes responsible for both immune and non-immune phenotypes. In our view, this notion necessitates that future studies examining the evolution of human gene expression and physiology explore what the term “immune” really means. If the immune system is so promiscuous as to be cross-referenced into such diverse functions as brain development, neurological function, placental implantation, spermatogenesis and bone development is it fair to describe a cytokine, for example, that is engaged in such pathways as “immune” alone? Though study areas concerned with evolutionary trade offs and life history frequently consider the energetic requirements of immunity vs other body systems, as a whole, human evolutionary biology and biological anthropology have not fully incorporated a view of the immune system as a consideration for the evolution of other physiological systems. If we are not considering the role genes cross-referenced into the immune system may play in the development and evolution of any other system the view of human and eukaryotic biology, broadly, is incomplete. We will also have a missed opportunity to reiterate the connectedness of human evolution to the extant human condition, and leverage studies concerned with human evolution to better inform fields associated with human health and immunity.

This is especially true in biological fields that readily interface with clinical research,

which tend to transact in the language of immunity even though the physiological compartment of focus is ostensibly not the immune system. It is critical that human evolution studies continue to develop in this manner as key characteristics of the human species appear to be strongly tied to the immune system. Human stature, for example, is connected to immune function, with body height associated B cell responses to infection, and pro-inflammatory cytokines slowing long bone development at the growth plate (Krams et al. 2014; MacRae et al. 2006; Sederquist et al. 2014). Human neurological function and behaviour appear inextricably tied to immune activity (Piccinni et al. 1998; Reaux-Le Goazigo et al. 2013; Sheridan and Murphy 2013), and evolutionary interactions with pathogens are hypothesized to affect current cytokine expression in mental health conditions such as depression and anxiety disorders (e.g. PATHOS-D hypothesis)(Raison and Miller 2013). The process of deep interdigitation of placentae, a factor that influences maternal mortality and is characteristic of humans and other catarrhines, appears to be influenced by immune gene expression (Benirschke and Kaufmann 2006; Capellini 2012; Crosley et al. 2013; Pudney et al. 2016; Yang et al. 2017). We posit that the genetic promiscuity of the immune system is such that its overlap with other body system makes it, at times, inseparable from other body functions. When considering what defines immune vs non-immune functions, it is also important that studies consider seemingly non-immune body components can be integral to the act of eliminating invading foreign matter or dying and cancerous cells. That many of our most famous examples of immune adaptation (i.e. HBS sickle cell alleles, Thalassemias) involve the canonical immune tissues and cells very indirectly suggests that maybe the descriptor “immune” can be very broadly applied to any genetic or physiological alterations which can impede invading micro-organisms or cancerous and dying cells, regardless of expressing tissue or component (Brinkworth 2017). Minimally, the promiscuity of “immune”

genes and the immune activities of non-immune tissues supports the assertive consideration of the immune system and the function of immunity in the evolutionary modeling of all other body systems.

Materials and Methods

Data Sources and Tissue Designations Data sources for expression analysis are listed in Tables S1 and S2, and are otherwise noted in text. For the purposed of our anaylses here kidneys, liver and brain were designated “non-immune” as they do not serve as major sites of leukocyte development and education, compared to colon, lymph nodes, spleen, thymus and whole blood/marrow which are key in the development and maintenance of cellular immunity.

RNA-Seq Mapping and Differential Expression (DE) Analyses RNA-Seq data was downloaded from the NHPRTR (Peng et al. 2015), with human RNA-Seq sequences for the same tissues obtained from the Illumina Body Map2 project (Derrien et al. 2012) stored by ENCODE, and three other studies of human tissues (Clark et al. 2015; Lin et al. 2014; Linsley et al. 2014) archived in the Genome Expression Omnibus. RNA isolation, library preparation for the NHPRTR data was completed by the same groups across species, and sequencing completed on the Hiseq2000. The human data was collected across projects and sequenced on Hiseq2000, with the exception of whole blood which was sequenced on a Illumina HiScanSQ. PCA analysis was completed on the complete dataset and no batch effects were noted for human (see Fig1, FigS1, FigS2). Fastq sequences were mapped to the species specific genome (hg38, panTro4, calJac3.2.1, rheMac8, and micMur1) using the STAR aligner (Dobin et al. 2013). Counts per orthologous genes for all tissues were generated using HT-Seq (Anders et al. 2015). Orthology

was assessed using Ensembl Genes 86 (Zerbino et al. 2018). Orthologous gene expression across the primate order are assessed in this study, as opposed to novel genes in a lineage, as this is a more conservative approach to understanding human immunity. Orthologs were identified via Biomart in the R environment (biomaRt) (Durinck et al. 2009; Zerbino et al. 2018). Gene expression was normalized using EdgeR (Lun et al. 2016; Robinson et al. 2010) and differential expression (DE) was assessed as minimum 20% fold change in expression at a false discovery rate (fdr) or 10% or less.

Signatures of Positive Selection in Protein-Coding Regions Dn/ds values from human, chimpanzee, and rhesus macaque were obtained from Ensembl using biomaRt (Durinck et al. 2009), with correlations performed in R, where $1 - dn/ds$ vs. p-values of DE were compared. Enrichment analyses were performed using Enricher (Chen et al. 2013; Kuleshov et al. 2016) and GOrilla/Revigo (Eden et al. 2009).

Specificity Scores Specificity scores were calculated as in (Haygood et al. 2007) and (Kosiol et al. 2008), and species-specific shifts in specificity as in (Babbitt et al. 2017). Briefly, the input values are the log Fold Change in Immune vs Non-Immune tissues. There is one distribution for each species, allowing for the species to species contrast. Here, 0=evenly expressed in tissues to 1= gene expression is specific to a given set of tissues.

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Table 1. Immune and Non-Immune Functions of the Top Ten Most Strongly Responding Genes to LPS in Human/Chimp/Rhesus Macaque Monocytes

LPS responding gene	Immune function (selected)	Other physiological system	Other functions
IL-6 *	Acute phase responses (Zilberstein et al. 1986) B-cell differentiation(Ikebuchi et al. 1987) Inflammation (Zilberstein et al. 1986) T17 and Treg cell development (Zheng et al. 2008)	CNS Musculo-skeletal Circulatory	Neurogenesis (Bauer et al. 2007; Deverman and Patterson 2009) neuronal function (Fann and Patterson 1994; Marz et al. 1998; Oh and O'Malley 1994) myokine/fat breakdown (Gudiksen et al. 2017; Wueest et al. 2014) Osteoclast activity(Ota et al. 1999; Ota et al. 2001) Angiogenesis (Wei et al. 2003)
CCL20	Cell trafficking of DC, T and B cells(Anderle et al. 2005; Ito et al. 2011) Antimicrobial activity towards <i>E. coli</i> (Hoover et al. 2002) Inflammation (Hromas et al. 1997)	Reproductive Genomic	Regulates sperm motility (Caballero-Campo et al. 2014; Diao et al. 2014) Induces DNA repair (Schwarz et al. 2002)
IL12B *	Th1 cell development (Cua et al. 2003) NK cell growth factor (Oppmann et al. 2000)	–	–
IL1A	Apoptosis (Hogquist et al. 1991) Fever generator (Cannon and Dinarello 1985) Antimicrobial activity towards <i>E. coli</i> (March et al. 1985)	reproductive Musculo-skeletal CNS	Spermatogenesis (Sarkar et al. 2008) Ovulation (luteal phase) (Cannon and Dinarello 1985) Osteoclast activity(Lord et al. 1991; Sabatini et al. 1988) Astroglial growth factor(Giulian et al. 1988)
TNFAIP6	Cell migration(Dyer et al. 2016; Dyer et al. 2014)	reproductive	Ovulation(Joyce et al. 2001; Ochsner et al. 2003)
GCH1 *	Response to LPS (Werner-Felmayer et al. 1993)	CNS	Dopamine synthesis (Duan et al. 2005)
TNF	Inflammation (Wu et al. 2011) Apoptosis (Obeid et al. 1993) Fever generator (Dinarello et al. 1986)	Respiratory Musculo-skeletal CNS	Lung morphogenesis (Zhu et al. 2007) Osteoclast differentiation (Komine et al. 2001) Smooth muscle proliferation (Barath et al. 1990; Ohta et al. 2005) Preserves synaptic strength (Beattie et al. 2002)
PDE4B *	Regulates INFg, IL-2 (Peter et al. 2007)	CNS	Mood regulation(O'Donnell and Zhang 2004)
G0S2	Apoptosis (Welch et al. 2009)	Growth/Metabolism	Lipolysis (Bednarski et al. 2016; Heier et al. 2015)
ELOVL7 *	-	Growth/Metabolism	Lipid metabolism and membrane development (Ohno et al. 2010)

* dn/ds = 0.01 - 0.85

Figure 2.

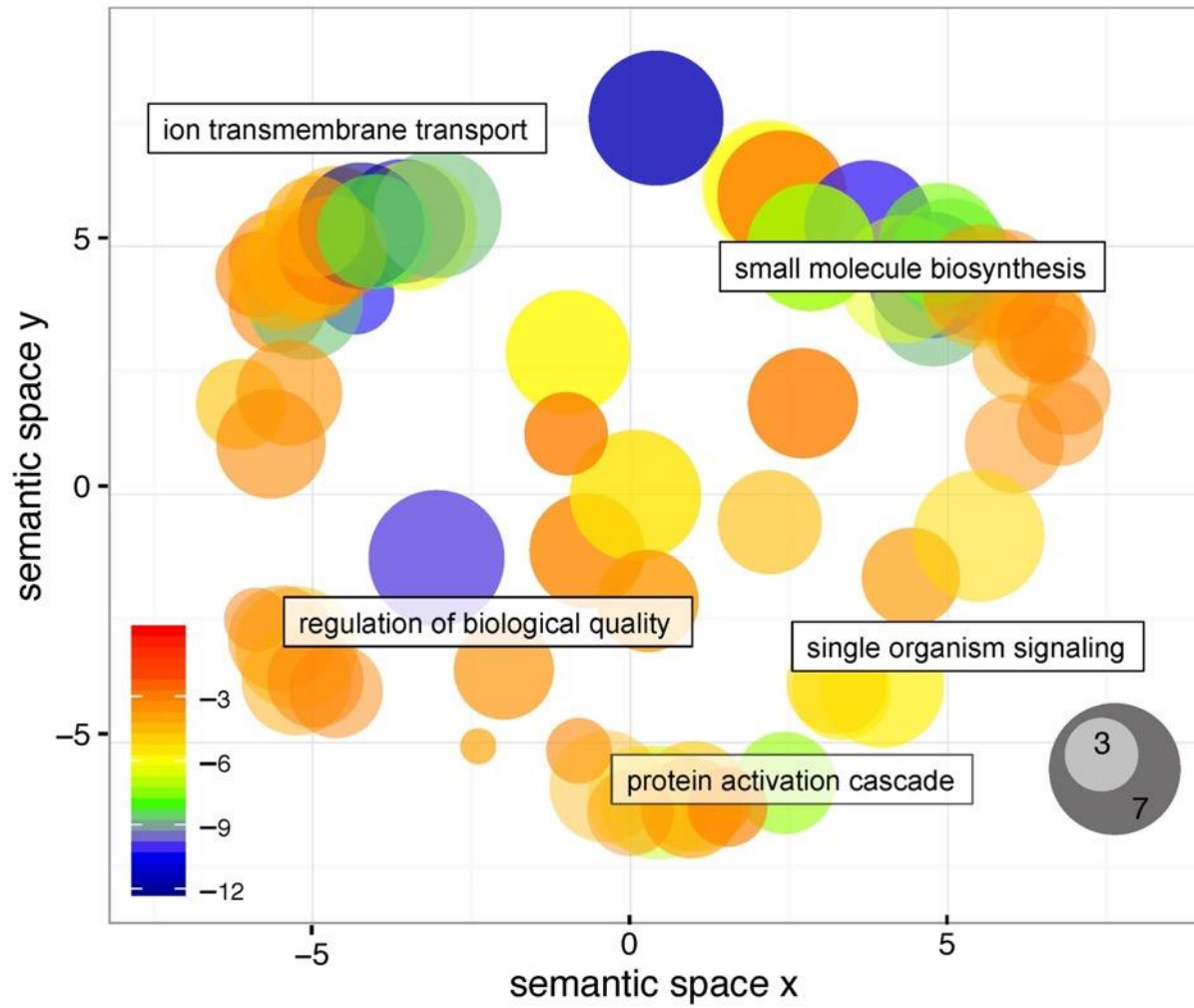


Figure 3.

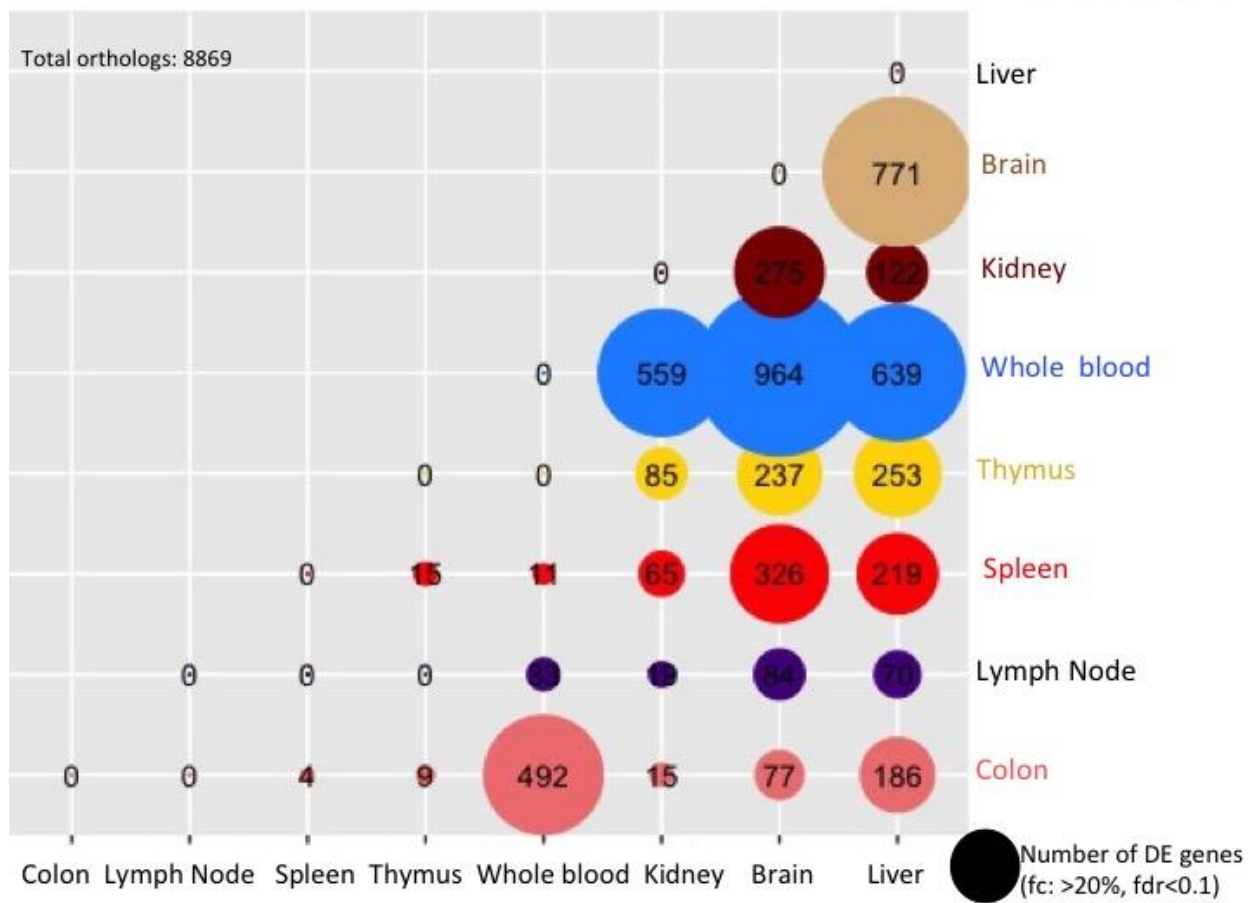
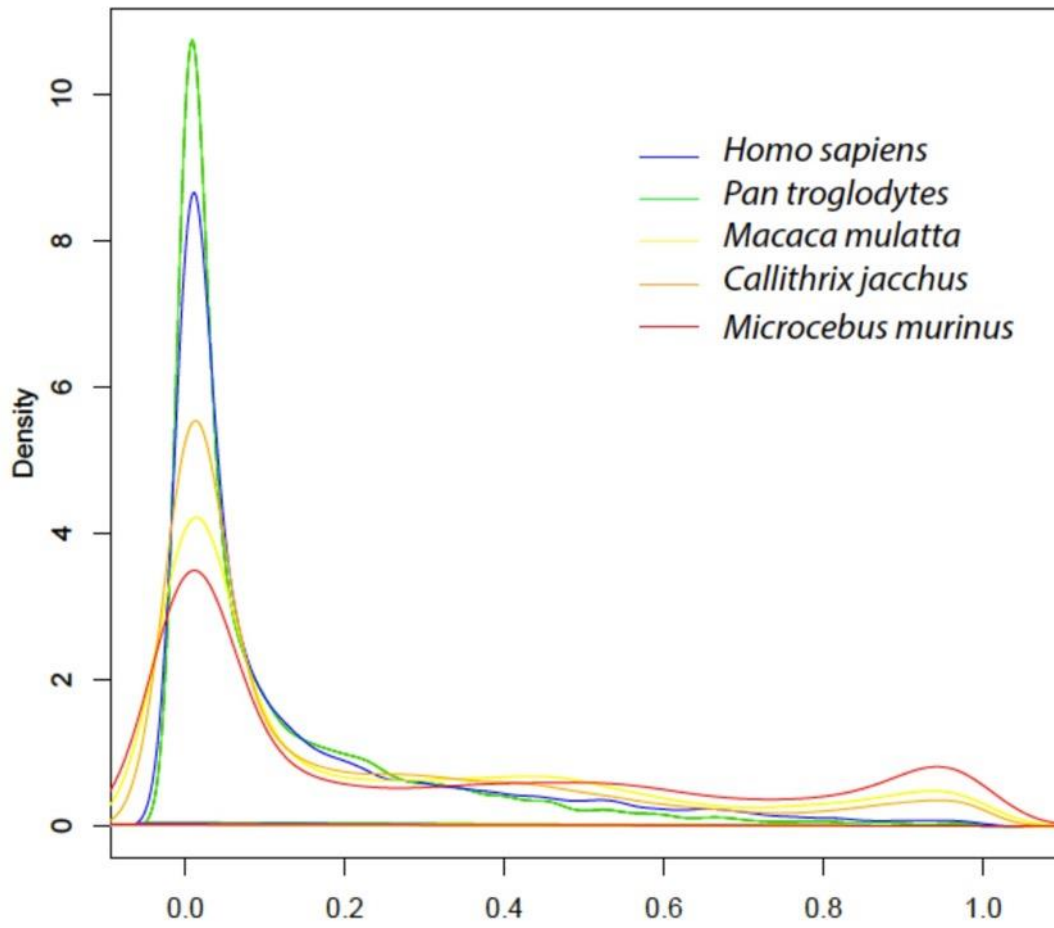


Figure 4.



Supplementary Figure S1.

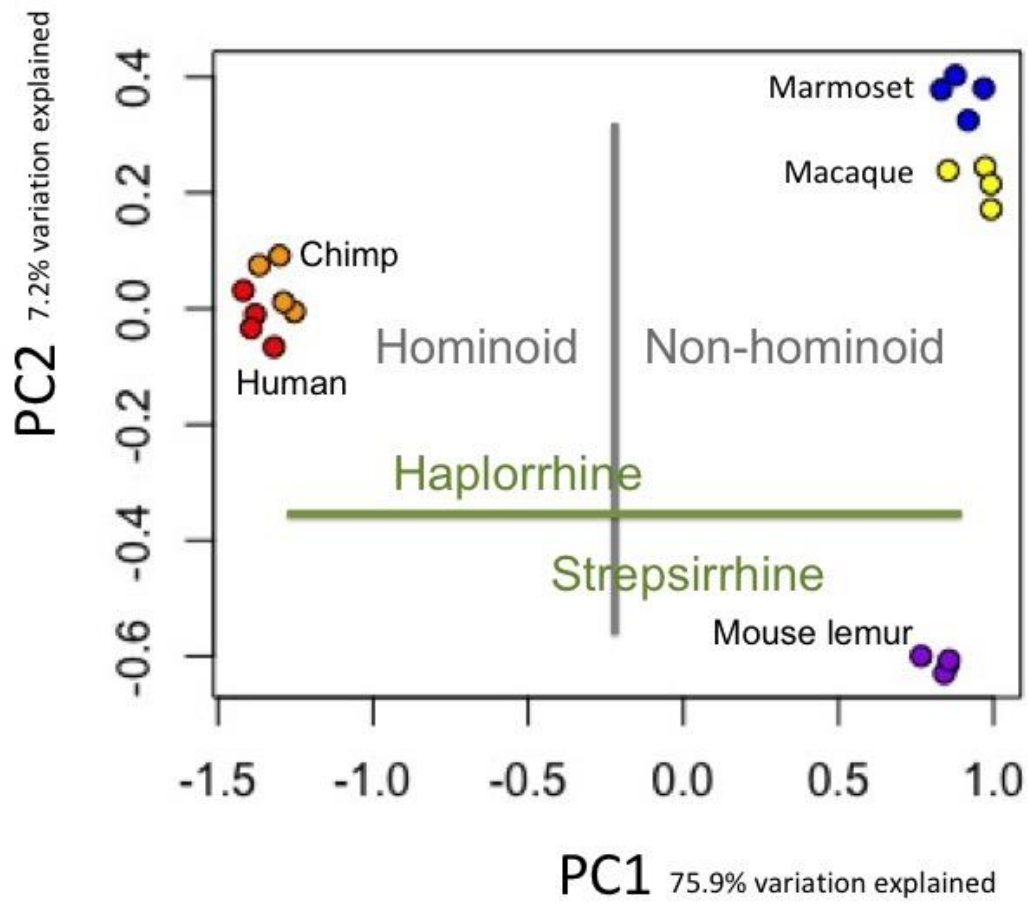


Figure Captions

Figure 1. Principal components analysis of baseline gene expression in 8 immune and non-immune tissues from 5 primate species. Brain, kidney, liver, were categorized as non-immune while colon, lymph node, whole blood, bone marrow, spleen and thymus were categorized as immune. Brain frontal lobe is denoted by (fr), temporal cortex (tc). PC1 and PC4 shown.

Figure 2. Overrepresentation analysis of GO Biological Processes in the Immune-NonImmune significantly differentially expressed gene list, in semantic space. Bubbles represent collapsed Gene Ontology terms (REVIGO) that Immune - Nonimmune DE genes populate. Bubble size indicates frequency that genes in the DE list populate a GO term in the underlying Gene Ontology (GOA) database. Bubble colour represents significance of GO term overrepresentation give the DE list and a background of 8868 genes, with increasingly cool colours indicating lower fdr values ($-1/\text{fdr}$). Bubbles are arranged in semantic space according to relatedness of the GO terms. Clusters represent GO categories of similar Biological Processes.

Figure 3. Number of differentially expressed (DE) genes between tissues. DE genes from pairwise contrasts between tissue types across primates (EdgeR) were assessed as genes with a fold change (fc) of greater than 20% and a false discovery rate (BH) of less than 0.1.

Figure 4. Density plot of specificity to either Immune or Nonimmune tissues over evolutionary time. In this analysis, the input values are the log Fold Change in Immune vs Non-Immune tissues. There is one distribution for each species, allowing for the species to species contrast.

The distributions represent specificity values (0 = shows even expression in all tissues to 1 = gene expression is specific to a given set of tissues).

Supplementary Figure S1. PCA of normalized expression values of tissues represented by each primate (kidney, brain, liver, spleen)

Supplementary Figure S2. PCA of normalized expression values of all tissues in study (brain, kidney, liver, colon, lymph node, whole blood, spleen, thymus, bone marrow).