Transcriptomics in T2-High Severe Asthmatics Treated with Biologics: Comparison between Anti-IgE, Anti-IL-5, and Anti-IL-5 Receptor Treatments

The development of monoclonal antibodies targeting key immunopathogenic processes has brought major advances in the treatment of severe Type 2 (T2)-high asthma (1). Although the target of each biologic is well understood, there is limited understanding of how biologic therapies alter the molecular milieu in the systemic compartment. This study aimed to further elucidate the mechanisms of action of mepolizumab, benralizumab, and omalizumab. We conducted a prospective study involving 35 patients diagnosed with severe T2-high asthma, in whom mepolizumab (n=9), benralizumab (n=14), or omalizumab (n=12) was initiated at the physician's discretion when patients fulfilled the Belgian reimbursement criteria. Patients underwent a comprehensive evaluation and blood sampling at baseline and after 16 weeks (omalizumab) or 24 weeks (mepolizumab and benralizumab) of treatment. We performed transcriptomic analysis to assess changes in blood inflammatory signals. The study was approved by the CHU Liege Ethics Committee (Reference no. 2005/181). (A detailed Methods section is provided in the data supplement.) For gene expression analysis, sequencing libraries were prepared with the Illumina Stranded Total RNA Prep, Ligation with Ribo-Zero Plus.

Mepolizumab and benralizumab improved asthma control and reduced exacerbations, along with decreasing blood eosinophil counts; benralizumab also reduced basophil counts. Omalizumab improved asthma control and reduced the exacerbation rate and fractional exhaled nitric oxide levels without affecting blood leukocyte counts (see Table E1 in the data supplement). In the mepolizumabtreated group, 147 genes were significantly downregulated, and 7 were significantly upregulated after 24 weeks of treatment compared with baseline. In the benralizumab-treated group, 165 genes were significantly downregulated, and 35 were significantly upregulated over the same period (Figures 1A and 1B). In total, 154 and 200 differentially expressed genes (DEGs) were identified after mepolizumab and benralizumab treatments, respectively (false discovery rate [FDR]–adjusted P values). It is interesting that 86 downregulated DEGs were common to both mepolizumab and benralizumab, primarily associated with eosinophils and basophils (see Table E2). However, benralizumab specifically modulated 114 genes, whereas mepolizumab specifically affected 68 genes. Only three statistically significant upregulated genes (CLASP2, BCR, and ZNF250) were highlighted with omalizumab after 16 weeks of treatment (Figure 1C). None of these genes overlapped with the DEGs identified in the mepolizumab- and benralizumab-treated groups. STRING analysis associated with the Markov clustering algorithm (or, MCL network clustering) (2) revealed that numerous downregulated genes after mepolizumab and benralizumab treatment were expressed in targeted biological processes such as "Granulocyte differentiation" (CEBPE, GATA2, and GATA1; Gene Ontology [GO]:0030851; FDR=1.43 10^{24}) and "Eosinophil fate commitment" (GATA2 and GATA1; GO:0035854; FDR=1.53 10^{23}) in the mepolizumab-treated group (see Figure E1) and "Basophil differentiation" (TAL1 and GATA1; GO:0030221; FDR=0.02) and "Eosinophil fate commitment" (GATA2 and GATA1; GO:0035854; FDR=0.02) in the benralizumab-treated group (see Figure E2). Another cluster of downregulated genes were part of the GO term "Purinergic nucleotide receptor signaling pathway" after mepolizumab therapy (P2RY14, P2RY10, P2RY2, and ADORA3; Figure E1) as well as benralizumab therapy (GPR34, P2RX6, P2RY2, and ADORA3; Figure E2) (GO:0035590; FDR=5.133 10^{26}). Gene set enrichment analysis (GSEA) revealed overrepresented reactome categories (Molecular Signatures Database, or MSigDB, v2023.1.Hs) that were enriched by genes expressed in pathways associated with IFN signaling and viral infection pathways after anti-IL-5 receptor therapies (Table 1; NES. 1.5; adjusted P value, 0.05). By contrast, both drugs were associated with the downregulation of reactome pathways involved in lipid metabolism and leukotriene synthesis (Table 1; NES, −1.5; adjusted P value, 0.05). In the case of omalizumab, we observed a reduced enrichment of pathways related to FceRI-mediated signaling (Table 1; NES,21.5; adjusted P value, 0.05), as well as a negative enrichment of pathways associated with various infectious diseases, including respiratory viruses such as influenza (Table 1; NES,22; adjusted P value, 0.05).

To the best of our knowledge, this study is the first to compare anti-IgE– and IL-5–targeted therapies in terms of transcriptomic effects. It demonstrates that inhibiting IL-5 with mepolizumab and targeting the IL-5 receptor with benralizumab produced notable alterations in the systemic immunoinflammatory network, whereas neutralizing IgE with omalizumab resulted in minimal impact. The evaluation of mepolizumab and benralizumab highlighted modulations primarily related to eosinophil and basophil biology. Eighty-six DEGs were downregulated by both mepolizumab and benralizumab, likely reflecting the significant reduction in blood eosinophil content, as indicated by decreased expression of SIGLEC8, PRSS33, and ALOX15. However, benralizumab specifically modulated 114 genes, whereas mepolizumab affected 68 genes, highlighting the distinct molecular actions of these two biologics. Some of the downregulated genes after benralizumab treatment were previously reported in a microarray study (3). STRING analysis showed a cluster of downregulated genes associated with the GO

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S.G. analyzed the data. S.G. and R.L. interpreted the data; S.G. and R.L. drafted the manuscript. F.B., C.D., F.S., and R.L. revised the manuscript critically for important intellectual content. All authors read and approved the final manuscript.

This letter has a data supplement available online

Artificial Intelligence Disclaimer: No artificial intelligence tools were used in writing this manuscript.

term “Purinergic nucleotide receptor signaling pathway” after both mepolizumab and benralizumab therapies. In fact, purinergic signaling has been implicated in lung and airway cell biology, and asthma has been shown to have aberrant purinergic signaling (4).

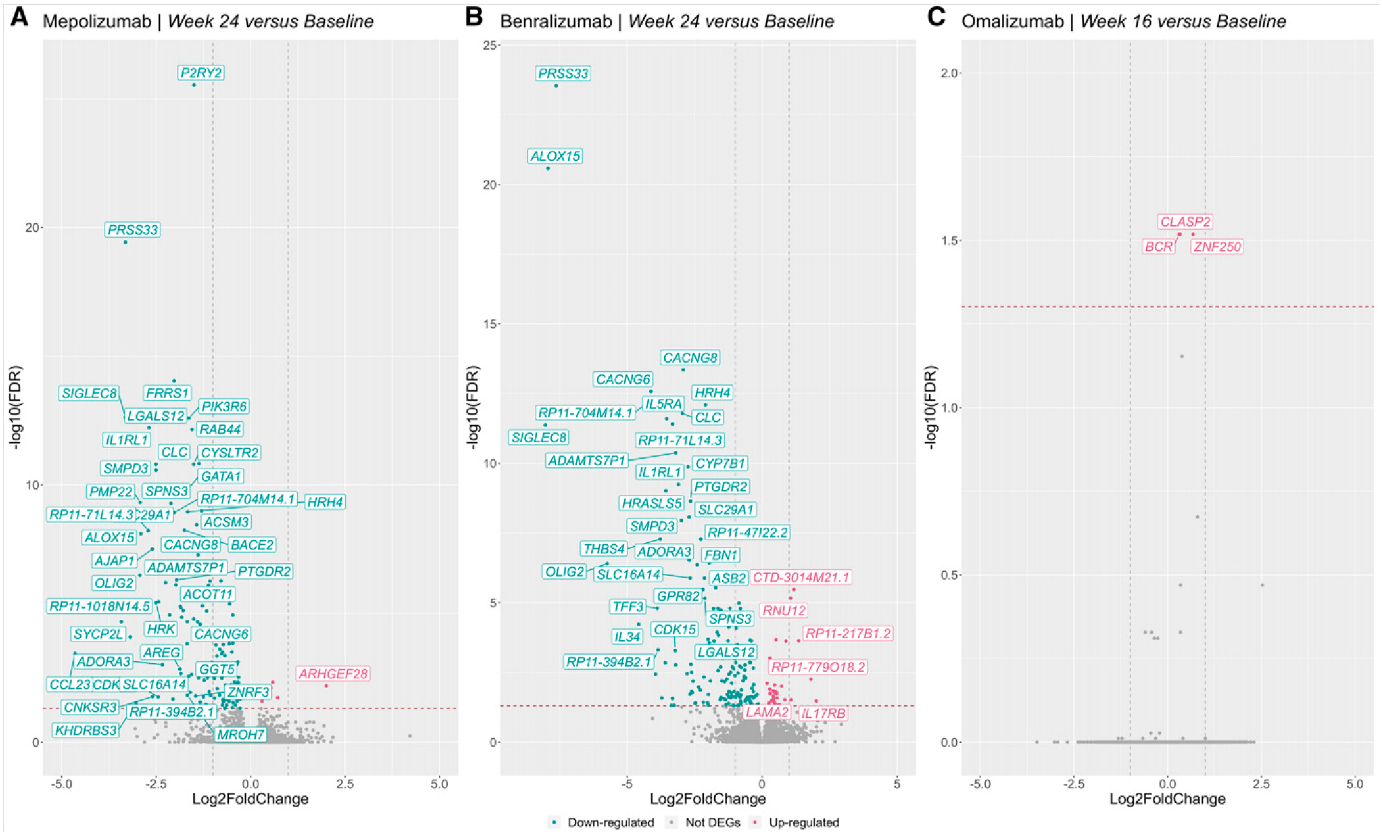

Figure 1. Volcano plots of the differences in transcriptomic profiles for the (A) mepolizumab, (B) benralizumab, and (C) omalizumab cohorts. DEGs = differentially expressed genes; FDR = false discovery rate.

Table 1. List of Significant Reactome Categories after GSEA comparing Week 16 or Week 24 of Mepolizumab, Benralizumab, or Omalizumab Treatment with Baseline

Significant Reactome Categories	Adjusted P Value	NES	Size
Mepolizumab Treatment at Week 24 versus Baseline			
REACTOME_SCAVENGING_OF_HEME_FROM_PLASMA	5.88 3 10^{27}	2.299	65
REACTOME_FCGR_ACTIVATION	5.88 3 10^{27}	2.282	67
REACTOME_INTERFERON_ALPHA_BETA_SIGNALING	5.88 3 10^{27}	2.265	66
REACTOME_CREATION_OF_C4_AND_C2_ACTIVATORS	2.72 3 10^{25}	2.213	63
REACTOME_CD22_MEDIATED_BCR_REGULATION	8.58 3 10^{25}	2.127	59
REACTOME_INITIAL_TRIGGERING_OF_COMPLEMENT	3.06 3 10^{25}	2.109	71
REACTOME_BINDING_AND_UPTAKE_OF_LIGANDS_BY_SCAVENGER_RECEPTORS	4.87 3 10^{25}	2.056	91
REACTOME_FCGR3A_MEDIATED_IL10_SYNTHESIS	2.58 3 10^{24}	1.983	91
REACTOME_ROLE_OF_LAT2_NTAL_LAB_ON_CALCIUM_MOBILIZATION	2.73 3 10^{23}	1.893	69
REACTOME_COMPLEMENT_CASCADE	2.97 3 10^{23}	1.831	97
REACTOME_ROLE_OF_PHOSPHOLIPIDS_IN_PHAGOCYTOSIS	1.69 3 10^{22}	1.773	80
REACTOME_ECM_PROTEOGLYCANS	1.90 3 10^{22}	1.749	62
REACTOME_FCERI_MEDIATED_MAPK_ACTIVATION	1.68 3 10^{22}	1.714	85
REACTOME_ANTIGEN_ACTIVATES_B_CELL_RECEPTOR_BCR_LEADING_TO_ GENERATION_OF_SECOND_MESSENGERS	1.88 3 10^{22}	1.709	84
REACTOME_PARASITE_INFECTION	6.84 3 10^{23}	1.702	113
REACTOME_INTERFERON_GAMMA_SIGNALING	1.68 3 10^{22}	1.696	93

REACTOME_FCERI_MEDIATED_NF_KB_ACTIVATION	8.53 3 10^{23}	1.669	133
REACTOME_ANTIGEN_PROCESSING_CROSS_PRESENTATION	4.45 3 10^{22}	1.622	101
REACTOME_INTERFERON_SIGNALING	1.93 3 10^{23}	1.612	247
REACTOME_IMMUNOREGULATORY_INTERACTIONS_BETWEEN_A_LYMPHOID_AND_ A_NON_LYMPHOID_CELL	1.60 3 10^{22}	1.584	184
REACTOME_CELL_SURFACE_INTERACTIONS_AT_THE_VASCULAR_WALL	1.46 3 10^{22}	1.575	177
REACTOME_POTENTIAL_THERAPEUTICS_FOR_SARS	2.40 3 10^{22}	1.570	152

Table 1. (Continued)

Significant Reactome Categories	Adjusted P Value	NES	Size
Mepolizumab Treatment at Week 24 versus Baseline			
REACTOME_ANTI_INFLAMMATORY_RESPONSE_FAVOURING_LEISHMANIA_ PARASITE_INFECTION	4.45 3 10^{22}	1.524	127
REACTOME_MITOTIC_G1_PHASE_AND_G1_S_TRANSITION	3.20 3 10^{22}	1.510	148
REACTOME_SIGNALING_BY_THE_B_CELL_RECEPTOR_BCR	4.23 3 10^{22}	1.498	163
REACTOME_RHOA_GTPASE_CYCLE	4.22 3 10^{22}	1.490	146
REACTOME_PROGRAMMED_CELL_DEATH	2.65 3 10^{22}	1.476	199
REACTOME_VIRAL_INFECTION_PATHWAYS	1.66 3 10^{23}	1.409	726
REACTOME_SARS_COV_INFECTIONS	1.89 3 10^{22}	1.370	445
REACTOME_CELL_CYCLE_MITOTIC	1.63 3 10^{22}	1.369	489
REACTOME_CYTOKINE_SIGNALING_IN_IMMUNE_SYSTEM	1.96 3 10^{22}	1.308	715
REACTOME_CELL_CYCLE	4.95 3 10^{22}	1.298	610
REACTOME_ADAPTIVE_IMMUNE_SYSTEM	2.65 3 10^{22}	1.283	790
REACTOME_METABOLISM_OF_RNA	3.57 3 10^{22}	1.273	702
REACTOME_INFECTIOUS_DISEASE	2.46 3 10^{22}	1.266	910
REACTOME_DEVELOPMENTAL_BIOLOGY	5.14 3 10^{23}	21.363	926
REACTOME_SIGNALING_BY_NUCLEAR_RECEPTORS	1.46 3 10^{22}	21.559	214
REACTOME_SIGNALING_BY_RECEPTOR_TYROSINE_KINASES	5.53 3 10^{24}	21.560	479
REACTOME_PHOSPHOLIPID_METABOLISM	1.89 3 10^{22}	21.586	187
REACTOME_G_ALPHA_I_SIGNALLING_EVENTS	8.53 3 10^{23}	21.593	254
REACTOME_METABOLISM_OF_LIPIDS	1.06 3 10^{25}	21.617	664
REACTOME_SIGNALING_BY_NTRKS	2.66 3 10^{22}	21.618	123
REACTOME_GPCR_LIGAND_BINDING	8.44 3 10^{24}	21.649	334
REACTOME_GLUCOSE_METABOLISM	3.24 3 10^{22}	21.678	84
REACTOME_CLASS_A_1_RHODOPSIN_LIKE_RECEPTORS	1.18 3 10^{23}	21.725	234
REACTOME_G_ALPHA_Q_SIGNALLING_EVENTS	5.53 3 10^{24}	21.820	175
REACTOME_NR1H2_AND_NR1H3_MEDIATED_SIGNALING	2.82 3 10^{22}	21.839	40
REACTOME_REGULATION_OF_LIPID_METABOLISM_BY_PPARALPHA	2.21 3 10^{23}	21.842	111
REACTOME_SIGNALING_BY_GPCR	5.42 3 10^{29}	21.843	559
REACTOME_INOSITOL_PHOSPHATE_METABOLISM	1.69 3 10^{22}	21.879	46
REACTOME_CREB1_PHOSPHORYLATION_THROUGH_NMDA_RECEPTOR_MEDIATED_ ACTIVATION_OF_RAS_SIGNALING	3.18 3 10^{22}	21.886	28
REACTOME_NR1H3_NR1H2_REGULATE_GENE_EXPRESSION_LINKED_TO_ CHOLESTEROL_TRANSPORT_AND_EFFLUX	3.20 3 10^{22}	21.897	33
REACTOME_SYNTHESIS_OF_LEUKOTRIENES_LT_AND_EOXINS_EX	1.81 3 10^{22}	21.901	18
REACTOME_SURFACTANT_METABOLISM	1.60 3 10^{22}	21.927	23
REACTOME_NUCLEOTIDE_LIKE_PURINERGIC_RECEPTORS	1.46 3 10^{22}	22.000	16
Benralizumab Treatment at Week 24 versus Baseline			
REACTOME_EUKARYOTIC_TRANSLATION_ELONGATION	6.15 3 10^{217}	2.647	93
REACTOME_EUKARYOTIC_TRANSLATION_INITIATION	1.28 3 10^{217}	2.642	119
REACTOME_RESPONSE_OF_EIF2AK4_GCN2_TO_AMINO_ACID_DEFICIENCY	3.96 3 10^{214}	2.513	101
REACTOME_REGULATION_OF_EXPRESSION_OF_SLITS_AND_ROBOS	7.76 3 10^{217}	2.492	165
REACTOME_NONSENSE_MEDIATED_DECAY_NMD	1.98 3 10^{213}	2.452	115

REACTOME_RRNA_PROCESSING	5.23 3 10^{217}	2.426	200
REACTOME_SELENOAMINO_ACID_METABOLISM	2.43 3 10^{211}	2.387	107
REACTOME_SRP_DEPENDENT_COTRANSLATIONAL_PROTEIN_TARGETING_TO_MEMBRANE	1.51 3 10^{211}	2.375	112
REACTOME_ACTIVATION_OF_THE_MRNA_UPON_BINDING_OF_THE_CAP_BINDING_COMPLEX_ AND_EIFS_AND_SUBSEQUENT_BINDING_TO_43S	8.31 3 10^{28}	2.305	60
REACTOME_CELLULAR_RESPONSE_TO_STARVATION	8.91 3 10^{211}	2.249	152
REACTOME_INFLUENZA_INFECTION	8.50 3 10^{211}	2.216	154
REACTOME_SIGNALING_BY_ROBO_RECEPTORS	4.50 3 10^{212}	2.207	208
REACTOME_SARS_COV_1_MODULATES_HOST_TRANSLATION_MACHINERY	2.88 3 10^{25}	2.171	37
REACTOME_TRANSLATION	1.13 3 10^{212}	2.169	276
REACTOME_RRNA_MODIFICATION_IN_THE_NUCLEUS_AND_CYTOSOL	4.13 3 10^{24}	1.974	58
REACTOME_SARS_COV_2_MODULATES_HOST_TRANSLATION_MACHINERY	6.42 3 10^{24}	1.969	51
REACTOME_ATTENUATION_PHASE	3.36 3 10^{23}	1.957	27
REACTOME_AUF1_HNRNP_D0_BINDS_AND_DESTABILIZES_MRNA	1.88 3 10^{23}	1.921	54
REACTOME_FORMATION_OF_FIBRIN_CLOT_CLOTTING_CASCADE	7.86 3 10^{23}	1.917	32
REACTOME_PROTEIN_HYDROXYLATION	1.92 3 10^{22}	1.871	17
REACTOME_HSF1_DEPENDENT_TRANSACTIVATION	9.58 3 10^{23}	1.834	34
REACTOME_HEDGEHOG_OFF_STATE	6.42 3 10^{24}	1.833	107
REACTOME_SARS_COV_1_HOST_INTERACTIONS	1.02 3 10^{23}	1.828	95

(Table 1. (Continued))

Benralizumab Treatment at Week 24 versus Baseline

REACTOME_THE_ROLE_OF_GTSE1_IN_G2_M_PROGRESSION_AFTER_G2_CHECKPOINT	3.66 3 10^{23}	1.805	73
REACTOME_METABOLISM_OF_POLYAMINES	6.43 3 10^{23}	1.802	58
REACTOME_HSF1_ACTIVATION	1.86 3 10^{22}	1.791	30
REACTOME_METABOLISM_OF_AMINO_ACIDS_AND_DERIVATIVES	2.54 3 10^{27}	1.788	338
REACTOME_COMPLEX_I_BIOGENESIS	8.87 3 10^{23}	1.774	57
REACTOME_SCF_BETA_TRCP_MEDIATED_DEGRADATION_OF_EMI1	1.13 3 10^{22}	1.770	54
REACTOME_DEGRADATION_OF_GLI1_BY_THE_PROTEASOME	1.07 3 10^{22}	1.761	58
REACTOME_RESPIRATORY_ELECTRON_TRANSPORT_ATP_SYNTHESIS_BY_CHEMIOSMOTIC_ COUPLING_AND_HEAT_PRODUCTION_BY_UNCOUPLING_PROTEINS	2.49 3 10^{23}	1.757	109
REACTOME_TNFR2_NON_CANONICAL_NF_KB_PATHWAY	2.99 3 10^{23}	1.744	99
REACTOME_ASYMMETRIC_LOCALIZATION_OF_PCP_PROTEINS	1.02 3 10^{22}	1.741	63
REACTOME_METABOLISM_OF_RNA	5.28 3 10^{211}	1.739	701
REACTOME_MET_PROMOTES_CELL_MOTILITY	3.79 3 10^{22}	1.734	38
REACTOME_RESPIRATORY_ELECTRON_TRANSPORT	3.36 3 10^{23}	1.731	102
REACTOME_GPER1_SIGNALING	2.83 3 10^{22}	1.722	40
REACTOME_CROSS_PRESENTATION_OF_SOLUBLE_EXOGENOUS_ANTIGENS_ENDOSOMES	2.63 3 10^{22}	1.719	48
REACTOME_DECTIN_1_MEDIATED_NONCANONICAL_NF_KB_SIGNALING	1.92 3 10^{22}	1.714	60
REACTOME_NEGATIVE_REGULATION_OF_NOTCH4_SIGNALING	2.85 3 10^{22}	1.690	53
REACTOME_SIGNALING_BY_HEDGEHOG	3.72 3 10^{23}	1.668	143
REACTOME_PCP_CE_PATHWAY	9.37 3 10^{23}	1.667	91
REACTOME_CHEMOKINE_RECEPTORS_BIND_CHEMOKINES	3.90 3 10^{22}	1.657	52
REACTOME_REGULATION_OF_RUNX3_EXPRESSION_AND_ACTIVITY	3.79 3 10^{22}	1.643	54
REACTOME_DEGRADATION_OF_AXIN	3.82 3 10^{22}	1.638	54
REACTOME_RESPONSE_TO_ELEVATED_PLATELET_CYTOSOLIC_CA2	9.58 3 10^{23}	1.618	119
REACTOME_REGULATION_OF_MRNA_STABILITY_BY_PROTEINS_THAT_BIND_AU_RICH_ELEMENTS	3.79 3 10^{22}	1.577	86
REACTOME_MAPK6_MAPK4_SIGNALING	3.79 3 10^{22}	1.573	82
REACTOME_SARS_COV_2_HOST_INTERACTIONS	6.16 3 10^{23}	1.566	185
REACTOME_VIRAL_INFECTION_PATHWAYS	1.07 3 10^{26}	1.552	726
REACTOME_NERVOUS_SYSTEM_DEVELOPMENT	2.57 3 10^{25}	1.550	538
REACTOME_SARS_COV_1_INFECTION	2.55 3 10^{22}	1.548	139
REACTOME_HOST_INTERACTIONS_OF_HIV_FACTORS	1.92 3 10^{22}	1.539	127
REACTOME_INTERFERON_SIGNALING	7.29 3 10^{23}	1.535	245
REACTOME_BETA_CATENIN_INDEPENDENT_WNT_SIGNALING	3.69 3 10^{22}	1.523	141
REACTOME_THE_CITRIC_ACID_TCA_CYCLE_AND_RESPIRATORY_ELECTRON_TRANSPORT	2.85 3 10^{22}	1.503	159
REACTOME_INFECTIOUS_DISEASE	9.55 3 10^{25}	1.426	910

REACTOME_SARS_COV_2_INFECTION	2.91 3 10^{22}	1.414	276
REACTOME_CELLULAR_RESPONSES_TO_STIMULI	2.61 3 10^{24}	1.408	718
REACTOME_PLATELET_ACTIVATION_SIGNALING_AND_AGGREGATION	4.20 3 10^{22}	1.400	239
REACTOME_DEVELOPMENTAL_BIOLOGY	3.25 3 10^{24}	1.371	928
REACTOME_CYTOKINE_SIGNALING_IN_IMMUNE_SYSTEM	4.12 3 10^{22}	1.243	719
REACTOME_NEUTROPHIL_DEGRANULATION	4.35 3 10^{22}	21.332	464
REACTOME_METABOLISM_OF_LIPIDS	6.15 3 10^{27}	21.628	658
REACTOME_FATTY_ACID_METABOLISM	5.77 3 10^{23}	21.643	158
REACTOME_PI_METABOLISM	9.58 3 10^{23}	21.712	82
REACTOME_PHOSPHOLIPID_METABOLISM	3.81 3 10^{24}	21.729	189
REACTOME_SYNTHESIS_OF_PIPS_AT_THE_PLASMA_MEMBRANE	1.66 3 10^{22}	21.754	52
REACTOME_INOSITOL_PHOSPHATE_METABOLISM	1.94 3 10^{22}	21.768	46
REACTOME_INTERLEUKIN_RECEPTOR_SHC_SIGNALING	2.28 3 10^{22}	21.834	26
REACTOME_SYNTHESIS_OF_LEUKOTRIENES_LT_AND_EOXINS_EX	1.27 3 10^{22}	21.856	18
Omalizumab Treatment at Week 16 versus Baseline			
REACTOME_REGULATION_OF_RUNX1_EXPRESSION_AND_ACTIVITY	3.77 3 10^{22}	1.799	17
REACTOME_INTERFERON_GAMMA_SIGNALING	3.47 3 10^{22}	1.618	93
REACTOME_NEUTROPHIL_DEGRANULATION	1.71 3 10^{23}	1.485	461
REACTOME_SIGNALING_BY_INTERLEUKINS	1.97 3 10^{23}	1.477	432
REACTOME_DISEASES_OF_SIGNAL_TRANSDUCTION_BY_GROWTH_FACTOR_RECEPTORS_ AND_SECOND_MESSENGERS	1.59 3 10^{22}	1.386	409
REACTOME_SIGNALING_BY_RECEPTOR_TYROSINE_KINASES	8.62 3 10^{23}	1.379	485
REACTOME_RHO_GTPASE_CYCLE	1.00 3 10^{22}	1.379	431
REACTOME_CYTOKINE_SIGNALING_IN_IMMUNE_SYSTEM	9.58 3 10^{23}	1.334	721
REACTOME_SIGNALING_BY_RHO_GTPASES_MIRO_GTPASES_AND_RHOBTB3	1.27 3 10^{22}	1.334	633
REACTOME_TRANSPORT_OF_SMALL_MOLECULES	3.61 3 10^{22}	1.307	632
REACTOME_HEMOSTASIS	1.57 3 10^{22}	21.337	594
REACTOME_VESICLE_MEDIATED_TRANSPORT	3.24 3 10^{23}	21.369	689
REACTOME_CELLULAR_RESPONSES_TO_STIMULI	2.38 3 10^{23}	21.382	716

Table 1. (Continued)

Omalizumab Treatment at Week 16 versus Baseline			
REACTOME_PROCESSING_OF_CAPPED_INTRON_CONTAINING_PRE_MRNA	4.81 3 10^{22}	21.396	276
REACTOME_METABOLISM_OF_RNA	2.65 3 10^{24}	21.439	701
REACTOME_INFECTIOUS_DISEASE	2.59 3 10^{27}	21.522	915
REACTOME_LEISHMANIA_INFECTION	2.86 3 10^{23}	21.559	211
REACTOME_SIGNALING_BY_ROBO_RECEPTORS	4.21 3 10^{23}	21.567	206
REACTOME_METABOLISM_OF_AMINO_ACIDS_AND_DERIVATIVES	3.37 3 10^{24}	21.568	336
REACTOME_RESPIRATORY_ELECTRON_TRANSPORT_ATP_SYNTHESIS_BY_CHEMIOSMOTIC_COUPLING_AND_HEAT_PRODUCTION_BY_UNCOUPLING_PROTEINS	1.44 3 10^{22}	21.593	109
REACTOME_IMMUNOREGULATORY_INTERACTIONS_BETWEEN_A_LYMPHOID_AND_ A_NON_LYMPHOID_CELL	7.83 3 10^{23}	21.600	184
REACTOME_SARS_COV_1_INFECTION	1.33 3 10^{22}	21.612	139
REACTOME_VIRAL_INFECTION_PATHWAYS	8.57 3 10^{29}	21.649	729
REACTOME_SARS_COV_INFECTIONS	2.78 3 10^{27}	21.706	448
REACTOME_FC_EPSILON_RECEPTOR_FCERI_SIGNALING	8.43 3 10^{24}	21.707	184
REACTOME_ACTIVATION_OF_THE_MRNA_UPON_BINDING_OF_THE_CAP_BINDING_COMPLEX_AND_EIFS_AND_SUBSEQUENT_BINDING_TO_43S	1.58 3 10^{22}	21.739	60
REACTOME_SARS_COV_1_HOST_INTERACTIONS	5.45 3 10^{23}	21.741	95
REACTOME_CHEMOKINE_RECEPTORS_BIND_CHEMOKINES	2.44 3 10^{22}	21.754	48
REACTOME_TRANSLATION	2.97 3 10^{26}	21.803	276
REACTOME_RRNA_PROCESSING	1.04 3 10^{25}	21.831	200
REACTOME_ANTI_INFLAMMATORY_RESPONSE_FAVOURING_LEISHMANIA_PARASITE_INFECTION	2.71 3 10^{25}	21.861	127
REACTOME_REGULATION_OF_EXPRESSION_OF_SLITS_AND_ROBOS	1.45 3 10^{25}	21.863	164

REACTOME_SARS_COV_2_MODULATES_HOST_TRANSLATION_MACHINERY	2.69 3 10^{23}	21.902	51
REACTOME_POTENTIAL_THERAPEUTICS_FOR_SARS	4.09 3 10^{26}	21.940	152
REACTOME_SIGNALING_BY_THE_B_CELL_RECEPTOR_BCR	1.58 3 10^{26}	21.947	164
REACTOME_FCGAMMA_RECEPTOR_FCGR_DEPENDENT_PHAGOCYTOSIS	7.81 3 10^{26}	21.957	140
REACTOME_FCERI_MEDIATED_NF_KB_ACTIVATION	3.30 3 10^{26}	21.988	134
REACTOME_CELL_SURFACE_INTERACTIONS_AT_THE_VASCULAR_WALL	6.65 3 10^{28}	22.050	178
REACTOME_PARASITE_INFECTION	4.98 3 10^{27}	22.100	114
REACTOME_FCGR3A_MEDIATED_IL10_SYNTHESIS	3.30 3 10^{26}	22.146	92
REACTOME_CELLULAR_RESPONSE_TO_STARVATION	4.94 3 10^{29}	22.157	151
REACTOME_COMPLEMENT_CASCADE	5.19 3 10^{27}	22.170	101
REACTOME_NONSENSE_MEDIATED_DECAY_NMD	1.50 3 10^{28}	22.201	115
REACTOME_EUKARYOTIC_TRANSLATION_INITIATION	8.57 3 10^{29}	22.231	119
REACTOME_FCERI_MEDIATED_CA_2_MOBILIZATION	1.62 3 10^{27}	22.236	85
REACTOME_BINDING_AND_UPTAKE_OF_LIGANDS_BY_SCAVENGER_RECEPTORS	2.81 3 10^{28}	22.273	94
REACTOME_SARS_COV_1_MODULATES_HOST_TRANSLATION_MACHINERY	1.08 3 10^{26}	22.293	37
REACTOME_INFLUENZA_INFECTION	4.36 3 10^{211}	22.296	155
REACTOME_FCERI_MEDIATED_MAPK_ACTIVATION	1.73 3 10^{28}	22.300	86
REACTOME_INITIAL_TRIGGERING_OF_COMPLEMENT	1.47 3 10^{27}	22.307	75
REACTOME_SELENOAMINO_ACID_METABOLISM	3.83 3 10^{29}	22.315	107
REACTOME_ROLE_OF_PHOSPHOLIPIDS_IN_PHAGOCYTOSIS	8.57 3 10^{29}	22.343	80
REACTOME_ANTIGEN_ACTIVATES_B_CELL_RECEPTOR_BCR_LEADING_TO_GENERATION_OF_ SECOND_MESSENGERS	1.10 3 10^{29}	22.391	85
REACTOME_ROLE_OF_LAT2_NTAL_LAB_ON_CALCIUM_MOBILIZATION	1.08 3 10^{28}	22.413	70
REACTOME_FCGR_ACTIVATION	4.94 3 10^{29}	22.419	68
REACTOME_SRP_DEPENDENT_COTRANSLATIONAL_PROTEIN_TARGETING_TO_MEMBRANE	4.86 3 10^{212}	22.459	112
REACTOME_CREATION_OF_C4_AND_C2_ACTIVATORS	3.69 3 10^{210}	22.493	67
REACTOME_RESPONSE_OF_EIF2AK4_GCN2_TO_AMINO_ACID_DEFICIENCY	1.47 3 10^{212}	22.531	101
REACTOME_SCAVENGING_OF_HEME_FROM_PLASMA	9.81 3 10^{212}	22.578	66
REACTOME_EUKARYOTIC_TRANSLATION_ELONGATION	4.61 3 10^{214}	22.615	93
REACTOME_CD22_MEDIATED_BCR_REGULATION	6.21 3 10^{214}	22.683	60

Definition of abbreviations: GSEA = gene set enrichment analysis; NES = normalized enrichment score. Data in the Size column indicate the number of genes involved in the single-gene set.

Because gene expression is a complex and coordinated process, identifying DEGs alone may not provide meaningful insights. GSEA addresses this by evaluating all genes, including those without significant individual changes. GSEA revealed overrepresented reactome categories associated with IFN signaling and viral infection pathways after anti-IL-5 and anti-IL-5 receptor therapies. Respiratory viral infections cause approximately 80% of asthma exacerbations, and regular inhaled corticosteroid therapy does not completely inhibit virus-induced exacerbations (5–7). Multiple lines of evidence suggest that eosinophilic airway inflammation is associated with impaired innate antiviral activity (8–11). Our data suggest that controlling eosinophilic inflammation with benralizumab and mepolizumab has the potential to restore antiviral immunity, which may contribute to a reduced rate of exacerbations. The three genes upregulated after omalizumab treatment—CLASP2, BCR, and ZNF250—were associated with asthma in genomic studies. CLASP2 has been linked to childhood asthma, eosinophil count, and atopic eczema (12), whereas BCR (13) and ZNF250 (14) have been associated with various asthma traits, including eosinophil count. GSEA revealed that omalizumab treatment may reduce FceRImediated signaling, including NF-kB activation. It is interesting that pathways related to respiratory viral infections were also downregulated. A reduction in NF-kB activation has similarly been observed after 6 months of treatment with mepolizumab and dupilumab (15). Overall, our data suggest that neutralizing circulating IgE with omalizumab has a limited impact on the systemic network. This supports the notion that omalizumab primarily acts by inhibiting airway mast cell activation and the subsequent release of inflammatory mediators and cytokines, such as IL-13, as evidenced by the reduction in fractional exhaled nitric oxide levels.

Our study has several limitations. First, the small sample size has hindered a meaningful analysis of the relationship between gene expression changes and clinical outcomes. Second, without a control group of healthy subjects, we cannot assess which gene signatures are dysregulated at baseline in asthmatics. Additionally, a control group of severe T2-high asthmatics who were not receiving biologics would have been useful; however, given that our omalizumab group showed very limited changes, it might serve as a de facto control group. Third, our study focused exclusively on blood samples, which may not fully capture the effects of biologic treatments within the airways. In summary, mepolizumab and benralizumab modify the systemic gene expression network with both shared and distinct effects, whereas omalizumab has minimal impact.

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