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Atopic Dermatitis, Urticaria and Skin Disease

Transcriptomic Analysis of Allergic Patch Test Reactions in Non-Atopic Patients: A Comparative Study Across Multiple Allergens

David Pesqué[1,2] | Evelyn Andrades[2,3] | Pau Berenguer-Molins[4] | Júlia Perera-Bel[4] | Miquel Clarós[5] | Marta Bódalo-Torruella[5] | Mònica González-Farré[6] | Fernando Gallardo[2,3] | Ramon M. Pujol[2,3] | Ana M. Giménez-Arnau[2,3]

[1]Department of Medicine, Universitat Autònoma de Barcelona (UAB), Barcelona, Spain | [2]Department of Dermatology, Hospital del Mar Research Institute, Barcelona, Spain | [3]Department of Medicine, Universitat Pompeu Fabra (UPF), Barcelona, Spain | [4]MARData, Hospital del Mar Research Institute (HMRIB), Barcelona, Spain | [5]MARGenomics, Hospital del Mar Research Institute (HMRIB), Barcelona, Spain | [6]Department of Pathology, Hospital del Mar Research Institute, Barcelona, Spain

Correspondence: David Pesqué (David.Pesque@autonoma.cat; pesquedavid@gmail.com) | Ramon M. Pujol (rpujol@psmar.cat) | Ana M. Giménez-Arnau (anamariagimenezarnau@gmail.com)

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Keywords: allergic | contact dermatitis | immune | patch test | transcriptomic

ABSTRACT

Background: Immune mechanisms underlying elicitation in allergic contact dermatitis (ACD) have yet to be fully elucidated. Previous studies have shown a double-faceted nature of ACD with both common biomarkers among different allergens and allergen-specific imprinting, albeit with discordance in terms of relevant pathways involved. Several factors, including co-existing atopic dermatitis, may influence immune reactions. We aim to characterize molecular signatures and their immune mechanisms of different relevant allergens (nickel, 2-hydroxyethylmethacrylate [2-HEMA], methylisothiazolinone [MIT], formaldehyde) in strong and extreme positive (2/3+) patch test reactions of patients without atopic dermatitis.

Methods: A transcriptomic analysis of 40 skin biopsies of ACD reactions (11 nickel, 10 MIT, 10 2-HEMA, 9 formaldehyde) and 19 controls (petrolatum-occluded skin) was performed using RNA sequencing. Differentially expressed genes (DEG) were assessed, and enriched functional pathways were obtained with an over-representation analysis for allergens.

Results: ACD molecular profiling revealed a strong, common imprinting of DEG among allergens versus controls ($n = 814$), with further partially shared DEG among allergens ($n = 664$) and allergen-specific DEG ($n = 430$). The most relevant shared pathways were associated with immune adaptive and innate responses. All allergens exhibited mixed effector immune responses, mainly type 1 and 3 immunity, and, to a lesser extent, type 2 immunity. Furthermore, partially shared and unique DEG were associated with further inflammatory pathways, particularly for nickel and 2-HEMA.

Conclusions: This study confirms shared ACD imprinting among different allergens and shared pathways' predominant role in ACD elicitation in patients without atopic dermatitis, alongside allergen-specific immune processes and mixed effector responses (type 1, 3 and 2).

Abbreviations: 2-HEMA, 2-hydroxyethylmethacrylate; ACD, allergic contact dermatitis; DEG, differentially expressed gene(s); IQR, interquartile range; logFC, log fold change; MIT, methylisothiazolinone; Th, T-helper; THEGL, testicular haploid expressed gene protein-like; Up-reg, upregulated.

Ramon M. Pujol and Ana M. Giménez-Arnau contributed equally as last authors.

1 | Introduction

Allergic contact dermatitis (ACD) is a common inflammatory condition due to a type IV delayed hypersensitivity response [1]. ACD diagnosis requires performing patch testing, which induces a reaction to the culprit sensitizer [2]. Different animal and human studies have investigated the various mechanisms involved in different phases of ACD. Mechanisms in the sensitization phase have been extensively characterized, whereas immune mechanisms underlying elicitation have yet to be completely elucidated [3]. With respect to elicitation, molecular studies have revealed a double-faceted nature of this condition [4–8]. The presence of common biomarkers and pathways among different allergens in both patch-induced ACD and clinical ACD lesions has been described, with potential application to distinguish ACD from other conditions, including irritant contact dermatitis [4, 6]. However, molecular profiling has also shown allergen-dependent immune responses that could contribute to a specific polarization induced by allergen [5]. The number of allergen-specific differentially expressed genes (DEG) may even exceed the number of shared ACD biomarkers [4, 5].

To date, there has been discordance in the immune transcriptomic pathways involved in ACD lesions, particularly in relation to adaptive immune responses [4, 5, 8]. Several factors, including comorbid skin conditions and reaction severity, may influence the immunological mechanisms in transcriptomic skin studies for ACD. In this regard, atopic dermatitis has been shown to attenuate ACD immune reactions with subsequently altered polarization in patch test studies [9]. Previous studies have demonstrated that more severe patch test reactions may be associated with specific pathologic characteristics [10, 11], changes in the phenotype of peripheral blood T cells [12], and detected more dysregulated genes [4].

The aim of this study is to characterize, at a transcriptomic level, strong and extreme positive patch-test-induced ACD reactions in patients without atopic dermatitis, using four clinically relevant allergens, including nickel, 2-hydroxyethylmethacrylate (2-HEMA), methylisothiazolinone (MIT), and formaldehyde. Furthermore, we aim to evaluate specific immune features of allergens in well-characterized ACD reactions.

2 | Methods

2.1 | Study Design

The study was conducted between May 2023 and May 2024. Patients with strong (2+) and extreme (3+) reactions [2] in patch tests to one of the four studied allergens were included. In the manuscript, strong and extreme reactions may be referred to as "severe" patch test reactions for legibility purposes. Exclusion criteria were a current diagnosis of concomitant atopic dermatitis [13] or disagreement in the interpretation (irritant vs. allergic) and/or grading of the reaction. The absence of atopic dermatitis was evaluated through a structured two-step process that included a short questionnaire designed to identify clinical features and relevant history suggestive of atopic dermatitis (based on the UKWP criteria [13]), and an extensive dermatological evaluation to find exploratory signs of atopic dermatitis. Participants meeting the UKWP criteria were excluded, and if the exam revealed further potential signs of atopic dermatitis, the atopic dermatitis status was reassessed with the Hanifin and Rajka criteria [14], ensuring that only individuals without clinical and exploratory evidence of atopic dermatitis were included in the analysis. Control samples were obtained from petrolatum-occluded skin of healthy volunteers without prior inflammatory skin conditions. All patients signed an informed consent to participate. The study was approved by the Institutional Review Board protocol (n°:2023/10837/I).

2.2 | Patch Test Procedure and Skin Samples

Allergens used for patch testing were nickel sulfate hexahydrate (5.0% petrolatum), MIT (0.2% aqua), 2-HEMA (2.0% petrolatum), and formaldehyde (2.0% aqua) provided by Chemotechnique Diagnostics (Vellinge, Sweden). Allergens were chosen based on the frequency of clinical reactions in our setting, their clinical relevance, and the fact that those were chemically unrelated. Per the European Society of Contact Dermatitis guidelines, patch testing was performed using Finn Chambers (8 mm, SmartPractice) on Scanpor tape (Vennesla, Norway) [2]. Two clinicians evaluated the reaction grading on day 4. If there were agreement on the reaction fulfilling the inclusion criteria (2+ or 3+) and consent was obtained from the patient, two 3 mm punch biopsies were performed. One biopsy was stored in RNAlater at 4°C, and RNA was extracted within 7 days after sampling. The other biopsy was stored in formalin and evaluated by the Dermatopathology team to confirm the presence of spongiotic features in the reactions. In control patients, one punch 3 mm biopsy on day 4 was obtained.

2.3 | RNA Sequencing

Total RNA was extracted from 3 mm skin biopsies that were previously incubated with RNAlater for a maximum of 1 week, with the Genelute mammalian total RNA kit from Sigma-Aldrich (cat. No. RTN350). Tissue was disrupted and digested in the lysis buffer with proteinase K (P4850, Sigma-Aldrich, St Louis, Missouri, United States) and 2-mercaptoethanol (M3148, Sigma-Aldrich, St Louis, Missouri, United States) incubating it for 10 min at 55°C. After washing and centrifugation several times to remove contaminants, RNA was eluted with 50 μL of elution buffer. Two commercial kits were tested. This kit obtained the best performance in total RNA recovery and integrity of the sample. The samples were used immediately or stored at −80°C for future use.

Samples were processed according to Truseq RNA Exome protocol from Illumina (Document #10000000039582 v01 March 2018) following steps from 1 to 6_Enrich DNA Fragments page 18. Afterward, samples were processed using Illumina RNA preparation with enrichment Tagmentation protocol (Document #1000000124435 v03 April 2021) following steps from 6_Normalize Library page 15 to the end of the protocol. Samples were enriched with Illumina Exome Panel and Unique Dual Index from NEBNext were used. The initial total RNA input for every sample was 100 nanograms measured with Qubit 4.0. Two hundred nanograms of each amplified sample was enriched in pools up to 3 samples, libraries

profiles were checked by 2100 Bioanalyzer. All samples amplified enough to continue with the process. Final enriched libraries were quantified by 2100 Bioanalyzer (DNA 1000 kit). Samples were sequenced in a NextSeq2000 from Illumina with an estimated output of 25 million reads/sample and a read length of 2x50bp.

2.4 | RNA Sequencing Data Analysis

Raw sequencing reads in the fastq files were mapped with STAR (v.2.7.8) [15]. Gencode release 41 was based on the GRCh38.p13 reference genome and the corresponding GTF file. The table of counts was obtained with the featureCounts function in the package subread (v.2.0.3) [16]. The differential gene expression analysis (DEG) was assessed with voom+limma in the limma package (v.3.54.2) [17]. A gene was considered a DEG if an observed difference in normalized read counts had a Benjamini-Hochberg adjusted p-value <0.05 and a logFC >1 in absolute value.

We kept genes having more than 10 reads in at least nine samples, leading to a total of 16,603 genes. Raw library size differences between samples were treated with the weighted "trimmed mean method" TMM [18] implemented in the edgeR package version 3.40.2 [19]. For the differential expression (DE) analysis, read counts were converted to log2-counts-per-million (logCPM) and the mean–variance relationship was modeled with precision weights using voom approach in limma package. The model was adjusted by age, sex, technician, and batch.

Universal enrichment (ORA) [20] implemented in the clusterProfiler package (v.4.6.0) was used to obtain enriched functional pathways [21]. ORA was performed over (i) the resulting allergen upregulated genes from the individual comparisons of allergen versus control (FA vs. Control, Ni vs. Control, 2-HEMA vs. Control, and MIT vs. Control), (ii) the shared allergen upregulated genes between the latter four comparisons, and (iii) the shared downregulated genes between the latter four comparisons (upregulated in control). Functional annotation was obtained based on the enrichment of gene sets belonging to the collection c5.bp from the Molecular Signatures Database (MSigDB), which contains gene sets derived from the Biological Process Gene Ontology (GO) (v.2023.2) [22]. Pathways were considered statistically significant when fulfilling a threshold of adjusted p-value <0.05. Detailed Figure methodology can be found in File S1. All analyses were performed under R version 4.2.2 (R-Core-Team 2022).

3 | Results

Data are available at GEO (n°GSE282562). The differential expression analysis of all DEG can be found in File S2.

3.1 | Cohort Characteristics

A total of 40 patients with strong or extreme patch test reactions were included, as well as 19 controls. The allergen group distribution was as follows: nickel ($n=11$), MIT ($n=10$), 2-HEMA ($n=10$), and formaldehyde ($n=9$). No significant age differences were found between the contact allergy group (median 45.5 years [IQR: 36.5–57.0]) and the control group (median 57.0 years [IQR: 39.0–61.0]) ($p=0.1$). There was a higher number of males in the control group versus all ACD groups ($p=0.048$). In terms of reaction severity, extreme (3+) reactions per allergen were as follows: 2-HEMA ($n=8$), nickel ($n=7$), MIT ($n=4$), and formaldehyde ($n=4$). Patients' demographics and patch test results are available in supplementary File S3 and controls' data in File S4. Furthermore, supplementary Figure S1 illustrates the studied reactions. Histopathological examination revealed that all reactions exhibited spongiotic features.

3.2 | Molecular Profiling in Severe Patch Test Reactions Reveals a Stronger Transcriptomic Imprinting of Common Differentially Expressed Genes

The transcriptomic analysis identified upregulated ($n=1434$) and downregulated ($n=1115$) DEG in ACD versus control patients. The total number of DEG varied among allergens when evaluating every single allergen against the control group. In this regard, 2-HEMA had the highest number of DEG (upregulated $n=1642$; downregulated $n=1589$), followed by nickel (upregulated $n=1511$; downregulated $n=1227$), MIT (upregulated $n=1208$; downregulated $n=667$), and formaldehyde (upregulated $n=1000$; downregulated $n=400$) (Figure 1).

When upregulated DEG between all allergens (versus controls) were compared, a high proportion of shared DEG was present among the four allergens ($n=814$). However, partially shared genes by three ($n=347$) or two allergens ($n=317$) remained lower. In addition, unique allergen-specific DEG ($n=430$) were found, being particularly abundant for nickel ($n=132$) and 2-HEMA ($n=245$). Furthermore, the comparison of the shared upregulated DEG ($n=814$) log2(CPM) expression between control, 2+, and 3+ reactions, independently of the allergen, was

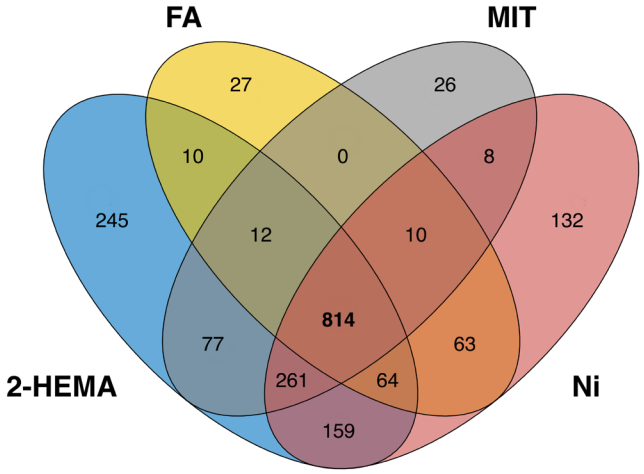

FIGURE 1 | Venn plot of upregulated differentially expressed genes in allergen versus control comparisons. The Figure considers a threshold of adjusted $p<0.05$ and log2(FC)>1.2–HEMA, 2–hydroxyethylmethacrylate; FA, formaldehyde; MIT, methylisothiazolinone; Ni, nickel.

significantly higher in 3+ reactions (Figure S2) when compared to both control and 2+ reactions.

3.3 | Previously Transcriptomic Core Signatures Were Identified in The Allergen Group

Previous works have suggested different combinations of DEG/protein sets that could delineate an ACD core signature [4, 6, 8]. File S5 presents the lists of genes/protein sets.

All previously defined core ACD DEG were found to be differentially expressed in most allergen samples. In this regard, a heatmap for each combination of genes (Figure S3a and S3b) showed a good qualitative performance in classifying subjects. In contrast, when evaluating the core protein ACD set, only two (CXCL10, CCL19) were upregulated, with all others being non-differentially expressed.

3.4 | Enriched Immune Pathways Are Frequently but Not Exclusively Linked to Type 1 Immunity in Severe Patch Test Reactions

Of the shared upregulated DEG among all allergens, important subsets of genes belong to the immune inflammatory response, including immune mediators and cellular responses (e.g., innate and adaptive immune responses). Among the top-150 DEG, according to logFC, several were chemokines (CXCL1, CXCL8, CXCL9, CXCL10, CXCL11, CCL3, CCL4, CCL8, CCL17, CCL19, CCL22), regulators of inflammatory mediators and interferons (MMP12, BATF, CD274, LTA, FASLG), and dendritic cell-related (CCR7, BATF3) and adaptive immunity-related genes (e.g., CD1B, CD8A, CTLA4, IL4Ll1, ITK, SLAMF1). In the group of top-150 DEG, several have been associated with type 1 immunity/Th1-related (CXCL9, CXCL10, CXCL11, CD80), type 3 immunity/Th17-related (PI3, SLAMF6), and type 2 immunity/Th2-related (CCL17, IL13, RSAD2) responses in previous works and/or the Biological Process Gene Ontology 2023 [5, 22]. Figures 2 and S4 illustrate the presence of type1, type 2, type 3 immunity and cytotoxicity DEG for allergens versus controls and the score comparison between allergens and controls. The comparison between allergens has shown differences in type 1 biomarkers between 2-HEMA and both nickel and formaldehyde, and in cytotoxicity biomarkers between 2-HEMA and formaldehyde. No differences could be found for type 2 and 3 immunity. File S6 details the complete list of shared ACD DEG.

The over-representation analysis (ORA) of the shared DEG resulting from the individual comparisons between allergens versus controls ($n = 814$; Figure 1) revealed 717/3013 significantly enriched pathways, most of which related to adaptive and innate responses. The top 25 enriched pathways were primarily related to adaptive immune pathways, particularly in association with T-cells (Figure 3). Several immune agents were frequently encountered in relation to the significantly enriched pathways. Figure 4 presents the three main pathways for the relevant immune agents in the allergen group, except for type 2 immunity, since only a unique enriched pathway was found. File S7 lists the enriched pathways and their associated genes.

3.5 | Upregulated Partially Shared DEG Show Further Common Immunologic Imprinting Among Different Allergens

The analysis of partially shared DEG (among two or three allergens) has overall shown that different allergen combinations may be associated with specific, partially shared DEG. Some combinations (e.g., formaldehyde and 2-HEMA, MIT and formaldehyde, or nickel and MIT) yielded no or few non-specific DEG. For all other combinations, DEG belonged to multiple immune, angiogenesis, and cell migration functions, among others (File S8). The combination of upregulated DEG between 2-HEMA with MIT and/or nickel revealed several specific DEG in relation to cytotoxicity and type 1 and 3 responses (IL-23A, IL6R, LOXL3). Moreover, Th2-related DEG (BCL-3, CCL-18) were also upregulated in the combined allergens comprising MIT, 2-HEMA, and formaldehyde. The combination of nickel and formaldehyde showed a different pattern of DEG, with most associated with the cell cycle; only very few were related to innate immunity or keratinization.

The ORA analysis of DEG from individual allergen-control comparisons (including both shared and non-shared DEG; Figure 1) revealed similar enrichment patterns of shared pathways across all allergens (Figure 5). Furthermore, this analysis revealed enriched partially shared immune pathways between allergens (Figure 5).

3.6 | Allergen-Specific Unique Molecular Responses Are Present in ACD and Show Some Variability According to Allergen

The analysis of allergen-specific molecular imprinting showed major involvement in the case of nickel and 2-HEMA. The ORA of the specific nickel DEG ($n = 132$; Figure 1) resulting from the comparison of nickel versus control revealed a transcriptomic signature related to gene cycle (e.g., mitotic nuclear division, chromosome segregation, mitotic nuclear division, among others) or immune response (e.g., cellular response to biotic stimulus, response to molecule of bacterial origin). As some gene cycle pathways have been associated with irritant responses [4, 6], the specific DEG for nickel were compared with ICD-associated genes from previous studies. A total of 26/132 specific DEG have been previously reported as irritant-related (e.g., AKR1B10, CDKN3, TYMS). The analysis of specific 2-HEMA DEG ($n = 245$; Figure 1) revealed that the specific transcriptomic expression involved a mixed inflammatory response composed of further type 2 (e.g., CCL11) and type 1 (e.g., IL18R1) DEG. In addition, specific pathways such as the regulation of the B-cell receptor signaling and the antigen-receptor mediated signaling were specifically enriched in this allergen. No specific pathway enrichment could be found for MIT and formaldehyde ($n = 26$ and 27; Figure 1). File S9 details the uniquely enriched pathways for nickel and 2-HEMA.

3.7 | Downregulated Shared DEG and Pathways Show Involvement of Skin Homeostasis

The common downregulated DEG resulting from the individual comparisons between allergens versus control ($n = 214$)

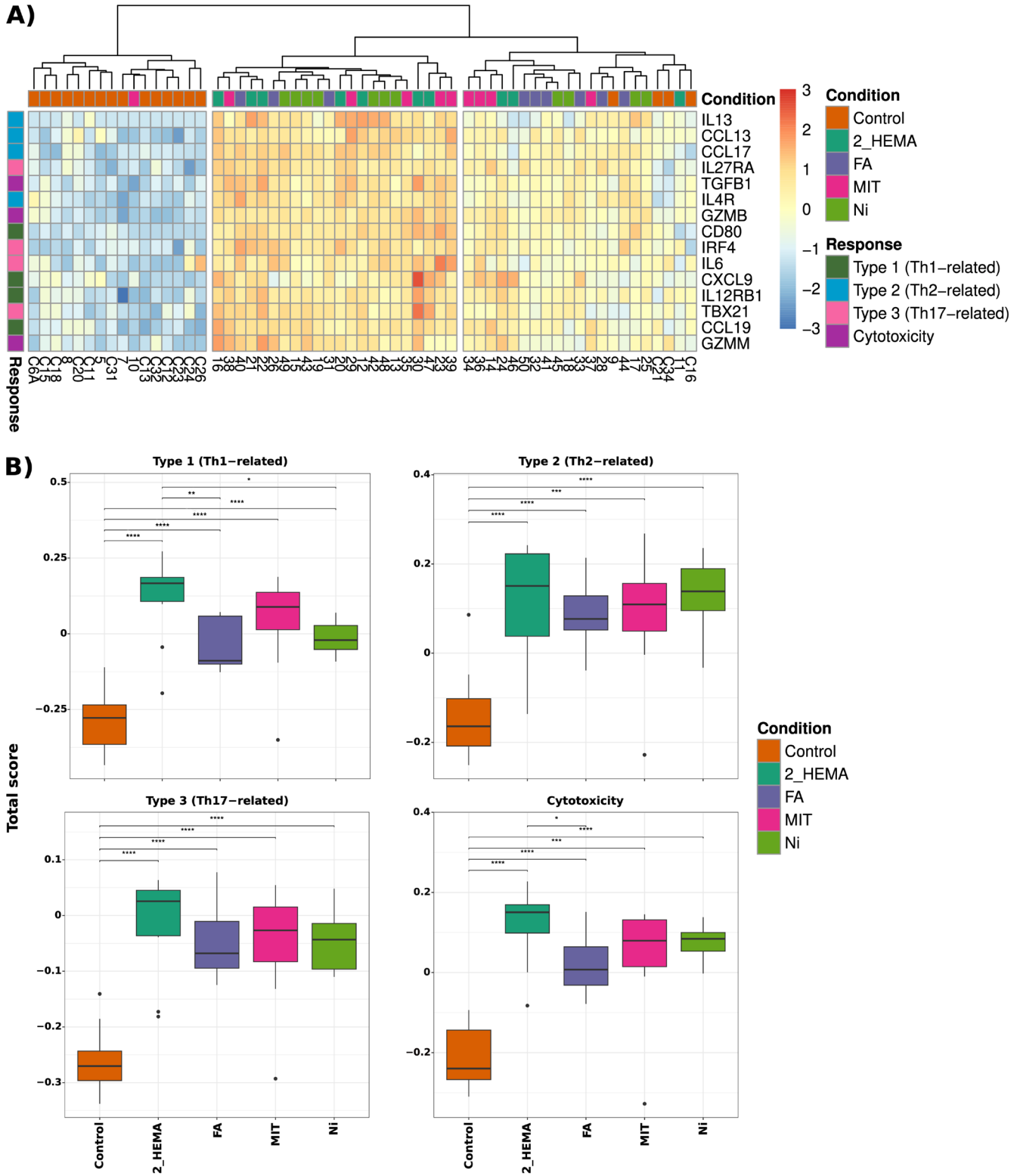

FIGURE 2 | (A) Heatmaps (Euclidean distance) of representative differentially expressed genes of type 1, type 2, type 3, and cytotoxicity show clusterization of allergens and (B) comparison of gene scores for these four categories between allergen groups and controls. Heatmaps illustrate the z-scores for the log2(CPM) expression values. The selected genes for each category arise from the shared upregulated DEG resulting from the individual allergen versus control comparison ($n = 814$). Rows and columns were clustered using Euclidean distance. Abbreviations: 2-HEMA, (2-hydroxyethylmethacrylate); FA, (formaldehyde); MIT, methylisothiazolinone; Ni, nickel. Interpretation of p-values in scores: (****$p \leq 0.0001$), (***$p \leq 0.001$), (**$p \leq 0.01$), and (*$p \leq 0.05$).

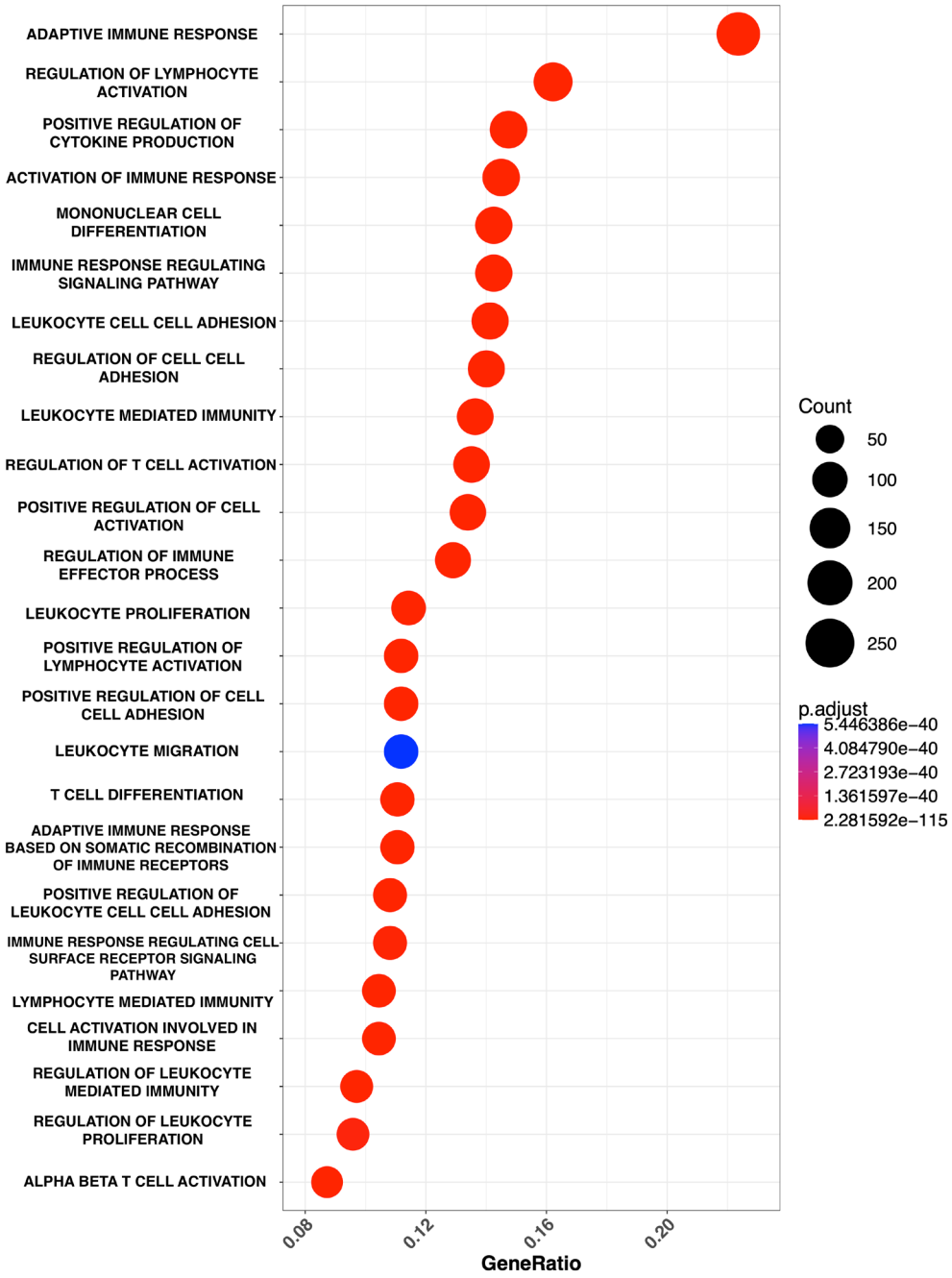

FIGURE 3 | Top-25 pathways according to the adjusted *p*-value in the allergen group. *p* adjust (adjusted *p*-value). Pathways were obtained from the ORA considering the shared differentially expressed genes ($n = 814$) resulting from the individual comparisons between allergens versus control (adjusted $p < 0.05$ & log2(FC) > 1).

(Figure 6A) corresponded mostly to epidermal proteins (e.g., LCE5A, LORICRIN, FLG2). This was reflected in the 14 significantly downregulated pathways for allergens versus controls, as shown in Figure 6B.

4 | Discussion

The main objective of our study was to characterize the transcriptomic features of severe patch-test ACD reactions to four clinically relevant allergens and evaluate their associated immune characteristics. Previous studies evaluated the transcriptomic imprinting of positive patch test reactions and probable allergic contact dermatitis clinical lesions [4–8]. Data have shown a double-faceted nature of this imprinting, not only with a "core ACD" imprinting but also with specific allergen molecular responses [4–6]. This study, focused only on strong and extreme allergic patch test reactions, has confirmed a common, strong ACD imprinting in a different variety of clinically relevant allergens. Furthermore, the ACD imprinting in terms of both common genes and pathways is higher than in previous studies evaluating ACD reactions. The absence of weak reactions in our study may suggest that in severe ACD there is a polarization towards core inflammatory pathways, at least in the late phase of elicitation (biopsies obtained at 96 h) of the studied allergens. Remarkably,

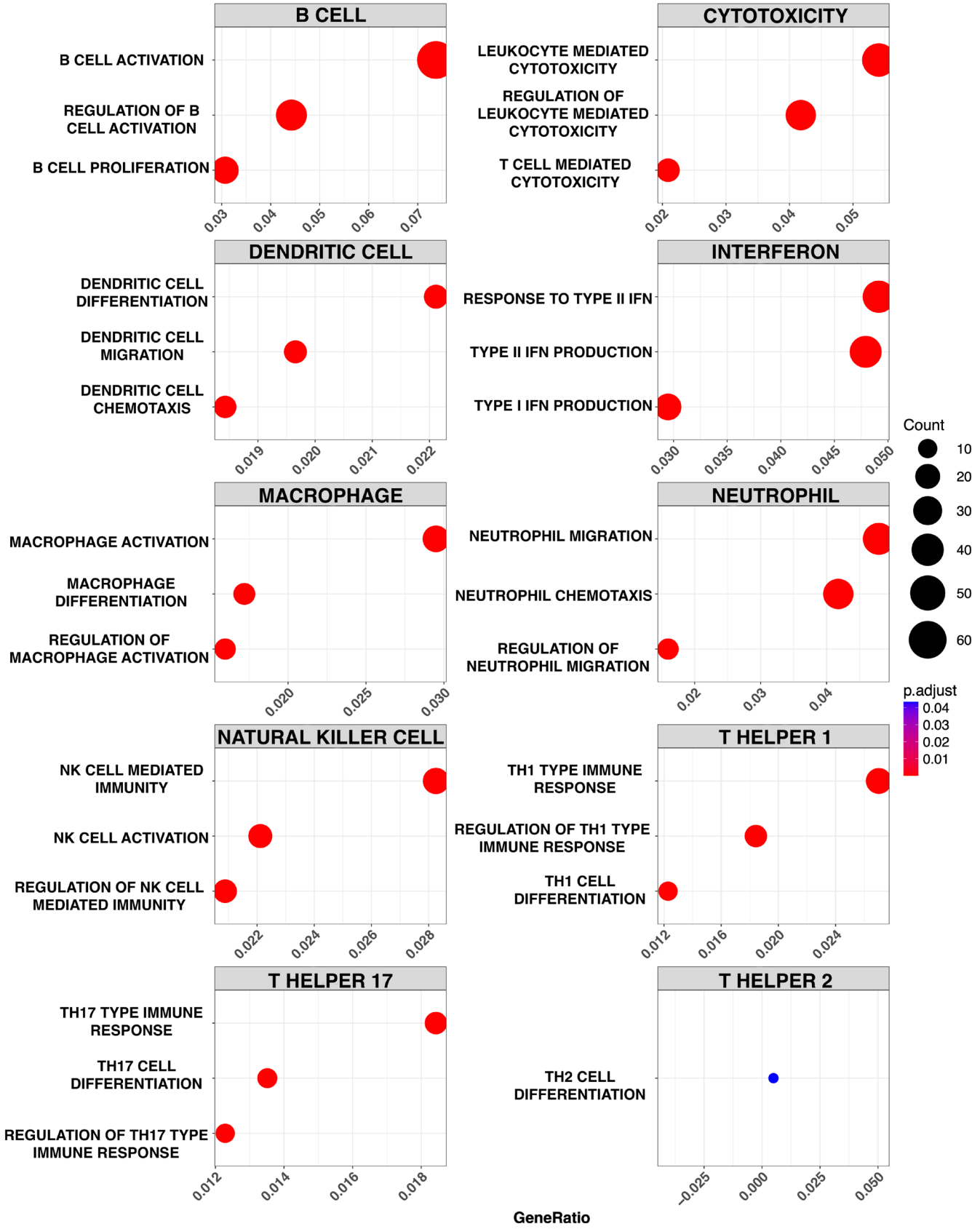

FIGURE 4 | Comparison of relevant enriched immune pathways for all allergens by main involved agents including cell types and interferon. *p* adjust (adjusted *p*-value). Pathways were obtained from the ORA considering the shared differentially expressed genes ($n = 814$) resulting from the individual comparisons between allergens versus control (adjusted $p < 0.05$ & $\log2(FC) > 1$). The specific DEG associated with each pathway are detailed in File S7.

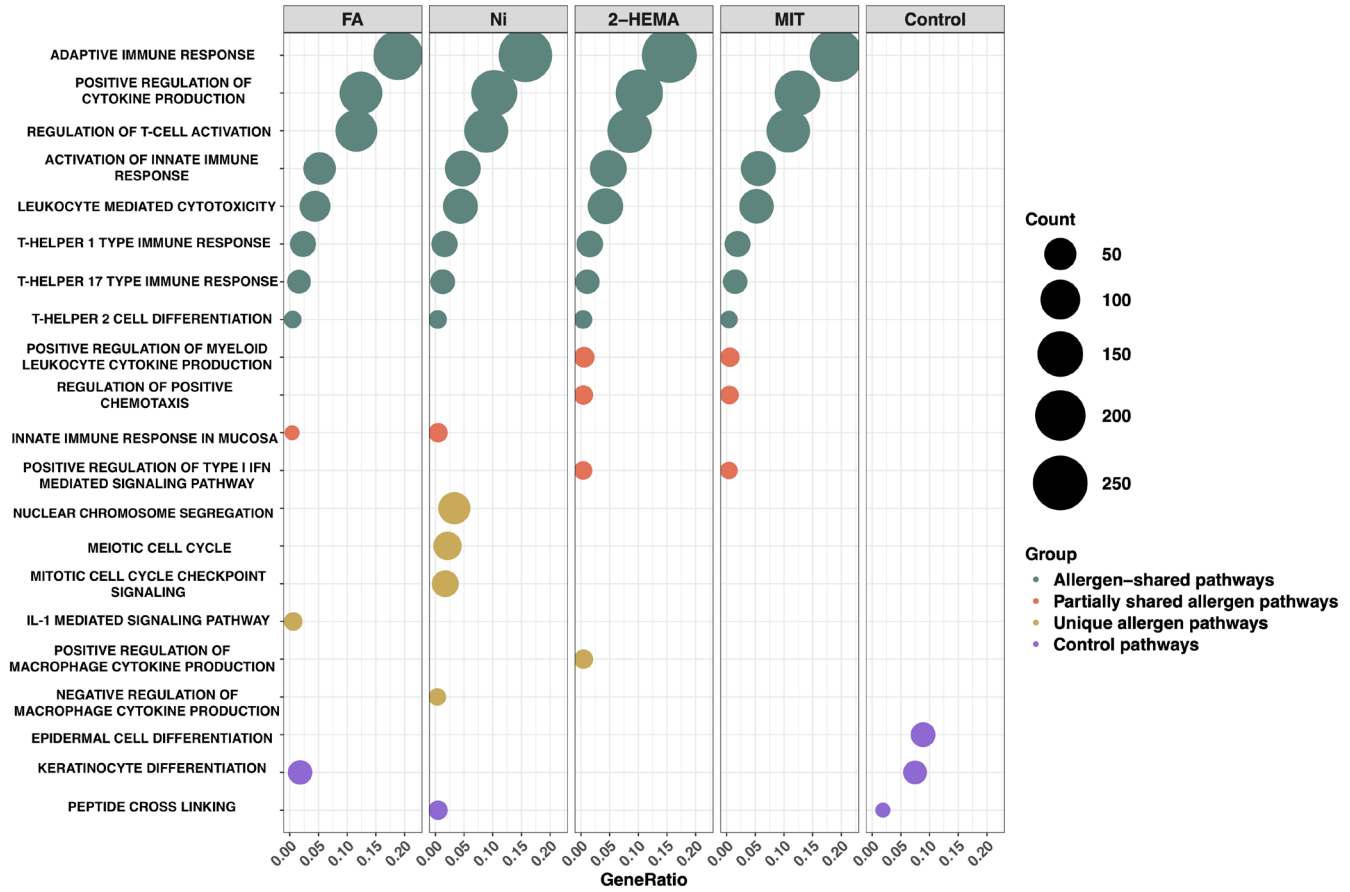

FIGURE 5 | Comparison of selected allergen and control pathways. Comparison of manually selected relevant pathways for the four allergens and controls. Allergen pathways (FA, Ni, 2-HEMA, and MIT) were obtained from the ORA considering the differentially expressed genes resulting from the individual comparisons between allergens versus control including shared and non-shared DEG (adjusted $p < 0.05$ & $\log2(FC) > 1$). Control pathways were obtained from the ORA considering the shared differentially expressed genes resulting from the individual comparisons between allergens vs. control (adjusted $p < 0.05$ & $\log2(FC) < -1$). All pathways fulfill a threshold of adjusted $p < 0.05$. The color legends for groups are specified in the legend as green color (allergen-shared pathways), orange color (partially shared pathways), golden color (unique allergen pathways), or pathways that were upregulated in the control group.

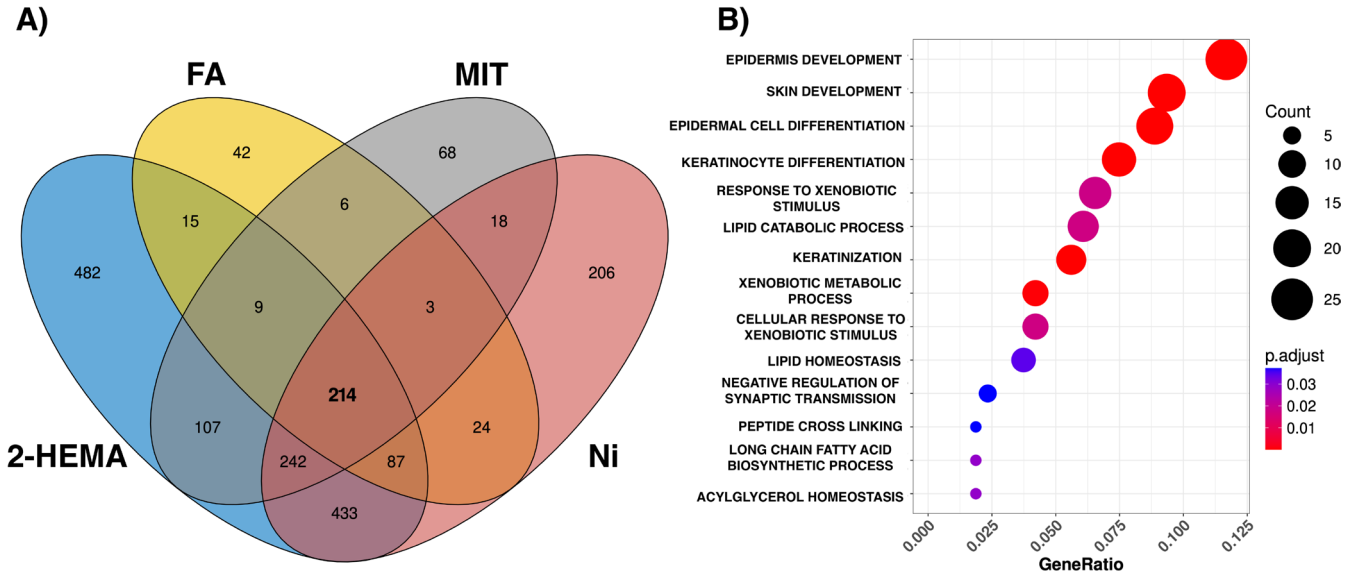

FIGURE 6 | Distribution of (A) upregulated differentially expressed genes and (B) enriched pathways in the control group (downregulated in the four allergens group). (A) Venn plot of downregulated differentially expressed genes in allergen versus control individual comparisons (adjusted $p < 0.05$ & $\log2(FC) < -1$). (B) Significantly enriched pathways in the control group (adjusted p-value < 0.05). 2-HEMA, 2-hydroxyethylmethacrylate; FA, formaldehyde; MIT, methylisothiazolinone; Ni, nickel, p adjust, adjusted p-value.

previously suggested core ACD transcriptomic imprinting has proven to perform well with our samples.

In regard to the common transcriptomic features of the ACD group, the evaluation of the immune response in these elicited reactions shows an important imprinting of innate and T-cell functions. Under the current perspective of ACD, skin inflammation (activation of keratinocytes and fibroblasts) as well as the innate immune response are essential in initiating and orchestrating the immune response during elicitation [23]. Murine models of contact hypersensitivity have shown the relevance of different immune actors in eliciting this condition. In this regard, dendritic cells, acting as allergen-presenting cells, may serve as a major source of key mediators that will promote the extravasation of more immune cells. Neutrophils, whose recruitment may accelerate and exacerbate the reaction [24], along with macrophages [25] and natural killer cells [26], are critical for the induction and development of contact hypersensitivity. With respect to interferon, type II interferon (including interferon gamma) has shown to be a critical part of the T-cell response and lead to further cytotoxic CD8 effects [27]. Type I interferons (alpha and beta interferons) may be an inflammatory response to counter the effect of interferon gamma [28]. Differential gene expression studies are important to understand the biology underlying ACD. In this regard, previous ACD "in vivo" transcriptomic studies have shown the occurrence of enriched pathways in relation to some of these immune agents. This study supports the contribution of innate immunity mechanisms and several immune cell groups (dendritic cells, neutrophils, natural killer cells, and macrophages) in the elicitation phase of "in vivo" ACD patch tests.

In terms of common adaptive immunity features, murine models of contact hypersensitivity have contributed to the understanding of B and T-cell roles. A multi-faceted nature has been suggested for B cells: the blockade of the B receptor contributes to the immune cleavage of the reaction, while B-cell depletion contributes to its worsening [29]. For T-cells, the role of CD8 and, to a lesser extent, CD4, in triggering the contact reaction is well-known [30, 31]. Transcriptomic "in vivo" human studies have confirmed the importance of B and T-cell imprinting in elicitation reactions [3, 4, 8]. However, variability in T-cell responses is present among studies. Previous studies agree on the presence of type 1/Th1 responses for all studied allergens, even though its presence in fragrance allergens may be smaller [5]. There is more heterogeneity in terms of other associated T-cell responses. In the case of nickel, ACD reactions have also been associated with type 3/Th17 [5] and type 2/Th2 [3, 4] immunity. Other allergens, including paraphenylenediamine, epoxy resin, methylchloroisothiazolinone/methylisothiazolinone, and squaric acid dibutyl ester, have shown an association between type 1/Th1 and type 2/Th2 responses [4, 8]. Fragrance allergens have been associated with a primarily type 2/Th2 response [5], even though only a few chemicals in a low number of patients have been studied. This study has found relevant leukocyte imprinting in the elicitation reaction across all allergens, including leukocyte-related cytotoxicity and Th-related responses. Our results do not show the degree of heterogeneity in the reaction polarization when compared to other studies. Instead, this investigation demonstrates a dominant type 1 immune response, closely associated with type 3, along with an accompanying less prominent type 2 immunity across all allergens. This study highlights the role of type 3 responses in eliciting ACD, likely due to its involvement of Th17-mediated innate and adaptive immunity [32].

Differences in Th/immune responses, compared to previous studies, may be attributed to the absence of atopic patients in this cohort, differences in studied allergens, and different timings for skin biopsies. Transcriptomic ACD studies in patients with atopic dermatitis have proven that results may present with halved interferon and type 1/Th1 pathways [9]. This, in turn, may confer more importance on other inflammatory pathways, leading to predominant type 2/Th2 responses. The knowledge of which T-cell pathways are altered in ACD is pertinent due to the discordance in therapeutic response to anti-Th2 therapies (e.g., dupilumab) in patients with ACD. As in previous studies [3, 4, 8], we have shown the enrichment of type 2/Th2 pathways occurs always within the context of other predominant immune actors (Th1, innate system, etc.). Thus, based on these results, anti-Th2 treatment might potentially target ACD pathways and interfere with patch testing; however, there remains discordance in the literature, both in terms of therapeutic response and implications for patch testing in general or for specific allergens that may involve more frequently this effector pathway [33–36].

This study reinforces that the presence of a common ACD signature may co-exist with the occurrence of specific allergen responses. Reasons for this variability are not well-known; however, likely factors could be intrinsic allergen properties/allergen reactivity [37, 38] and a differential interaction with epidermal amino acids/peptides [39]. To illustrate this point, nickel may directly interact with Toll-like receptors and activate the immune response [40]. In this context, our study showed that only the nickel-induced transcriptomic profile was associated with pathways enriched for Toll-like receptor signaling, such as those involved in the response to bacterial molecules [41]. Nevertheless, it is not certain whether the differential activation between allergens is responsible for subsequent immune differences. Among the unique enriched pathways per allergen, 2-HEMA was associated with specific B-cell responses, whereas nickel had specific innate responses and cell cycle processes. Data were lacking with respect to the transcriptomic characteristics of ACD to 2-HEMA, but there is evidence of strong induction of adaptive responses to acrylates [42]. As it relates to nickel, previous studies have detected "irritant-like" patterns (e.g., cell cycle and DNA repair related) [3] in ACD patch test lesions. In our study, despite the strong ACD imprinting, some irritant pathways were observed. As nickel contact allergy has been associated with filaggrin deficiencies [43] and there is a higher association of irritancy with a disrupted skin barrier [44], the association of contact allergy with some accompanying degree of irritancy could be a possible phenomenon in severe patch test/clinical lesions of ACD. Furthermore, previous histologic or transcriptomic studies have highlighted the possibility of both conditions co-existing in one reaction [45, 46].

The study of the downregulated genes and pathways for allergens revealed the involvement of genes and pathways related to the structural/homeostatic factors of the skin barrier. Remarkably, some pathways (e.g., peptide cross-linking, epidermal cell differentiation, keratinocyte differentiation) [4] have been described

as upregulated in irritant contact dermatitis studies. Among the most relevant downregulated DEG related to skin barrier (LCE5A, LORICRIN, FLG2), some have been shown to either inversely vary with high levels of inflammation (e.g., FLG2) [47], be downregulated by Th2 cytokines (e.g., LORICRIN) [48], or be reduced in other inflammatory conditions with epidermal involvement (e.g., LCE5A) [49]. Interestingly, among the two upregulated DEG previously associated with epidermal injury (CXCL8 and CCL17) [50, 51], CXCL8 was upregulated in the allergen group. Thus, severe ACD reactions, with evident epidermal involvement, may be associated with a reduction in skin barrier proteins and subsequent alteration in epidermal homeostatic functions.

This study presents a certain number of limitations. It was carried out on a small number of samples and using a limited number of allergens. Further studies involving a bigger subset of allergens are needed to clarify the immune imprinting of elicitation.

In conclusion, this study confirms the existence of a shared ACD imprinting and pathways across different allergens in definite ACD-induced patch lesions, which represent a significant aspect of the elicitation reaction. Furthermore, it reinforces the importance of different innate and adaptive immune responses during ACD elicitation, particularly type 1 but also type 3, and even type 2 responses, and may help to clarify the relevant immune pathways in the elicitation reaction of ACD in patients without atopic dermatitis. Further studies are needed to assess the immune mechanisms of other allergens (e.g., fragrances) and differential immune mechanisms in patients with atopic dermatitis.

Author Contributions

All authors meet criteria for authorship.

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Ethics Statement

The study was approved by the Institutional Review Board protocol (2023/10837/I). Patients gave written informed consent to publication of their details.

Conflicts of Interest

D.P. has received research funding from LEO Foundation. AGA is or recently was a speaker and/or advisor for and has received research funding or participated in research from Almirall, Amgen, AstraZeneca, Avène, Blue Print, Celldex, Escient Pharmaceuticals, Genentech, GSK, Harmonic Bio, Incyte, Instituto Carlos III–FEDER, Jaspers, Leo Pharma, Menarini, Mitsubishi Tanabe Pharma, Novartis, Sanofi–Regeneron, Septerna, Servier, Thermo Fisher Scientific, Uriach Pharma/Neucor, outside the submitted work. All other authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are openly available in GEO, reference number GSE282562.

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Supporting Information

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