Immunodeficiency, autoimmunity, and increased risk of B cell malignancy in humans with *TRAF3* mutations

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Abstract

Tumour necrosis factor receptor-associated factor 3 (TRAF3) is a central regulator of immunity. TRAF3 is often somatically mutated in B cell malignancies, but its role in human immunity is not defined. Here, in five unrelated families, we describe an immune dysregulation syndrome of recurrent bacterial infections, autoimmunity, systemic inflammation, B cell lymphoproliferation and hypergammaglobulinaemia. Affected individuals each had monoallelic mutations in *TRAF3* that reduced TRAF3 expression. Immunophenotyping showed patients' B cells were dysregulated, exhibiting increased NF-κB2 activation, mitochondrial respiration, and heightened inflammatory responses. Patients had mild $CD4^+$ T cell lymphopenia, with reduced naïve T cell proportions but increased regulatory T cells and circulating T follicular helper cells. Guided by this clinical phenotype, targeted analyses demonstrated that common genetic variants, which also reduce *TRAF3* expression, are associated with risk of B cell malignancies, systemic lupus erythematosus, higher immunoglobulin levels, and bacterial infections in the wider population. Reduced TRAF3 conveys disease risks by driving B cell hyperactivity via intrinsic activation of multiple intracellular pro-inflammatory pathways and increased mitochondrial respiration, with a likely contribution from dysregulated T cell help. Thus, we define monogenic *TRAF3* haploinsufficiency syndrome, and demonstrate how common *TRAF3* variants impact upon a range of human diseases.

One-Sentence Summary

Reduced TRAF3 in humans results in B cell hyperactivity and T cell defects predisposing to infections, autoimmunity and malignancy.

Main Text:

INTRODUCTION

Inborn Errors of Immunity (IEI) often present with a complex clinical picture including predisposition to infection, autoimmunity and malignancy. The genetic investigation of monogenic IEIs has provided unique insights into how individual genes control human immunity, and how their perturbation gives rise to pathology, and can both inform treatment of individual patients and provide a valuable resource for drug target discovery (*1*, *2*). In parallel, population-wide studies of the impact of common genetic variants on disease susceptibility often implicate immune genes. Integrating rare and common variant genetic studies to understand the impact of individual genes on immune function at the population level represents an opportunity for the field.

Tumour necrosis factor alpha receptor-associated factor 3 (TRAF3) is one of a family of cytoplasmic proteins that control signal transduction from different receptor families, including the tumour necrosis factor receptors (TNFRs) and Toll-like receptors (TLRs). TRAF3 serves as a constitutive negative regulator of the alternative NF-κB pathway, and has a potential role in many immune cell types, but defects in it have only been studied in detail in cell lines and mouse models (*3*). While genome-wide association studies (GWAS) have linked common variants at the *TRAF3* locus with several immune traits and autoimmune and atopic conditions (*4*–*8*), and the *TRAF3* variant p.R118W (rs143813189-T) has been reported in association with herpes simplex encephalitis in a single case (*9*), TRAF3's role in humans is yet to be fully determined. Here we describe the breadth of impact, and mechanism of action, of rare and common genetic variants in *TRAF3* on human immunity.

RESULTS

Patient clinical phenotypes consisting of infection and autoimmunity

We investigated nine individuals from five unrelated families with childhood-onset immune diseases and recurrent infections (**Fig. 1A and Table 1**). All patients had suffered recurrent ear and sinopulmonary infections, including pneumonias from encapsulated bacteria *Streptococcus pneumoniae* and *Haemophilus influenza*, resulting in early-onset bronchiectasis in several (**fig. S1A-E**). Patients A1 and E1 both underwent left lower lobectomy with histology showing bronchiectasis and follicular bronchiolitis with associated massive lymphoid hyperplasia, with a prominent lymphocytic infiltrate comprising T cells ($CD4^+ > CD8^+$), B cells and numerous plasma cells (**Fig. 1B and fig. S1F**). Lymphadenopathy was observed in multiple patients and lymph node histology showed granulomatous inflammation with no pathogens identified. Elective splenectomy due to massive splenomegaly was performed in patient B3, and histology showed numerous reactive lymphoid follicles with large germinal centers. T and B cell immunohistochemistry indicated normal follicular architecture was preserved with no evidence of abnormal clonality (using kappa/lambda staining), malignancy or extramedullary hematopoiesis (**Fig. 1C**). Immune dysregulation was prevalent; three patients were diagnosed with Sjögren's Syndrome and suffered sicca symptoms, each with anti-Ro52+ antibodies and characteristic B cell hyperplasia (**fig. S1G**). In addition to their Sjögren's Syndrome, patient B4 developed an ANCA-positive vasculitis with glomerulonephritis which was treated with cyclophosphamide and rituximab, resulting in persistently absent B cells. Immune dysregulation was also prominent in patient D1, with substantial atopic disease, multiple food and drug allergies, elevated serum IgE, as well as autoimmune thyroid disease, and an autoinflammatory phenotype of systemic juvenile inflammatory arthritis with markedly raised ferritin and IL-18, managed with IL-1 receptor blockade. Patient E1 first presented in infancy with recurrent

sinopulmonary bacterial infections and pneumonia, complicated by empyema, leading to bronchiectasis. Diagnoses of systemic autoimmunity and follicular bronchitis led to immunosuppressive therapy with prednisolone, hydroxychloroquine, and thalidomide, with concurrent antimicrobial treatments. Long term antimicrobial prophylaxis, immunoglobulin replacement, and lobectomy improved the patient's infection burden (**Supplementary patient case summaries**).

TRAF3 haploinsufficiency in patients

Genomic investigation revealed heterozygous premature stop-codon mutations in *TRAF3* in all patients, segregating with disease consistent with a pattern of autosomal dominant inheritance (**Fig. 1E**). In A1 the *de novo* single nucleotide change c.1275C>G in *TRAF3* resulted in the premature stop-gain p.Y425X, and in affected individuals of family B the nucleotide deletion c.1066Gdel caused the frameshift p.S356PfsX in *TRAF3*. Patients C1 and C2 each had a four nucleotide insertion in *TRAF3,* c.339_340TAGA, resulting in p.Q114X stop-gain mutation, and D1 had a *de novo* c.487C>T causing the stop-gain mutation p.R163X in *TRAF3.* In patient E1, as in patient A1, the *TRAF3* variant c.1275C>G p.Y425X was identified (**Fig. 1F)**. Each mutation caused a reduction of both *TRAF3* RNA and protein expression, consistent with haploinsufficiency (**Fig. 1G, H**). TRAF3 protein undergoes constitutive ubiquitination and proteasomal degradation via TRAF2-cIAP1/2, with reduction in TRAF2 or cIAP1/2 resulting in increased TRAF3 (*10, 11*). We hypothesized that the observed >50% reduction of TRAF3 protein may be a consequence of increased ubiquitin-mediated proteasomal degradation of residual TRAF3 from the wild-type allele due to a reduced ratio of TRAF3:TRAF2-cIAP1/2. Consistent with this, treatment with a proteasome inhibitor (MG132) increased TRAF3 expression to nearer the expected 50% of control (**fig. S2**). Therefore, in these families with evidence of an autosomal dominant inherited immune

dysregulatory syndrome, genetic investigation identified heterozygous high-impact *TRAF3* mutations resulting in *TRAF3* haploinsufficiency (*TRAF3*^{HI}).

Dysregulation of immune cells in *TRAF3*^{HI} patients

The features of this syndrome suggested widespread immune dysregulation. Total and CD4$^+$ T cell numbers were reduced in *TRAF3*^{HI} patients, with reduced naïve cells and increased proportions of memory T cells (**Fig. 2A-C and fig. S3A**). As observed in T cell-specific *Traf3*-deficient mice (*12*), regulatory T cells (T_{reg})were increased in *TRAF3*^{HI} patients (**Fig. 2D and fig. S3B**). CD8$^+$ T cell numbers were preserved, but the proportion of naïve CD8$^+$ T cells was reduced (**Fig. 2E,F** and **fig. S3C**). T cell proliferation in response to anti-CD3/CD28 was normal (**fig. S3D**), but there was reduced production of interleukin (IL)-17 and interferon (IFN)-γ following CD3/CD28 stimulation of patient T cells (**Fig. 2G**). Assessment of additional cytokines showed production of the T-helper (T_H)2 cell cytokines IL-4, IL-13, and IL-10 was comparable with controls (**Fig. 2G**), as was IL-2 (albeit in a single patient: **fig. S3E**). Reductions in IFN-γ and IL-17 but preserved T_H2-associated cytokines are consistent a mild impairment of T cell receptor signaling strength in *TRAF3*^{HI} patients, as was observed in T cell-specific *Traf3*-deficient mice (*13*), resulting in a relative increased propensity to T_H2 differentiation (*14*). Circulating T follicular helper (cT_{FH}) cells were increased in *TRAF3*^{HI} patients (**Fig. 2H**), with increased T_{FH} effector mRNA levels and surface ICOS expression (**Fig. 2I,J**). Circulating T follicular regulatory (cT_{FR}) cells were reduced (**Fig. 2K**), as also seen in T_{reg}-specific *Traf3*-deficient mice (*15*). Together increased T_{FH} and reduced T_{FR} would be likely to promote aberrant germinal center responses and immunoglobulin production . Therefore, a distinct pattern of T cell abnormalities was present in *TRAF3*^{HI} patients, which might support exaggerated B cell responses.

Polyclonal increased serum immunoglobulins were seen in most *TRAF3*^{HI} patients, but responses to polysaccharide vaccines were impaired and auto-antibodies were prevalent (**Table 1**).Total B cell numbers were normal (**Fig. 3A**), yet the proportion of class-switched memory cells was reduced across both IgG and IgA isotypes (**Fig. 3B, C**), while CD19^{+}CD21^{low}CD38^{-}Tbet^{high} B cells, associated with autoimmune disease (*16*), were increased (**Fig. 3D**). *TRAF3*^{HI} patients had normal numbers of circulating marginal-zone like B cells, and marginal zones appeared histologically normal in one patient (**Fig. 1C**), unlike mice (see (*17*) and Discussion). Patients had increased antibody-secreting cells in blood (**Fig. 3E**), and their B cells showed increased proliferation and induction of plasmablast formation upon stimulation with TLR-9 agonists and IL-2 (**Fig. 3F**). In patient-derived B lymphocytoblastoid cell lines (BLCLs), both TLR-7 and TLR-9 expression was increased, consistent with a more pronounced activation state than in control BLCLs, and TLR-9 stimulation resulted in enhanced production of TNFα (**fig. S4).** B cell receptor (BCR) repertoire analysis showed increased usage of IGHV4-34 and IGHV4-59, immunoglobulin heavy chain genes previously associated with SLE, vasculitis, Sjögren's syndrome, and lymphoma (**fig. S5A-E**) (*18, 19*). Thus, the clinical and immune phenotypes of patients with *TRAF3*^{HI} were consistent with an immune dysregulatory syndrome involving T cells and hyperactive and auto-reactive B cells.

We then explored myeloid cells in *TRAF3*^{HI} patients, as *Traf3*$^{flox/flox}$/*LysM*^{cre} myeloid cell-specific *Traf3*-deficient mice showed increased inflammatory cytokines after TLR stimulation in macrophages, and increased T-dependent antibody responses (*20*). Monocyte populations varied between patients (A1, B1, C1), with a possible increase of non-classical inflammatory (CD14^{dim}CD16^{++}) monocytes (**fig. S6A**). Circulating dendritic cells in *TRAF3*^{HI}

patients were broadly comparable to controls and, in the one patient where functional studies were possible, enhanced TNFα and IL-6 was observed in monocytes and dendritic cells (**fig. S6B-D**). These data raise the possibility that a dysregulated myeloid compartment may contribute to the clinical phenotype of *TRAF3*^{HI} patients.

Functional impacts in *TRAF3* haploinsufficient B cells

To understand the molecular mechanisms underlying the *TRAF3*^{HI} syndrome, we focused on B cells, given the prominence of B cell hyperactivity in patients, and on the nuclear factor-kappa beta 2 (NF-κB2) pathway, shown to be negatively regulated by TRAF3 in B cell malignancies (*21*). *TRAF3*^{HI} patient-derived BLCLs showed increased NF-κB inducing kinase (NIK) protein, phosphorylated NF-κB2 p100, and the active NF-κB2 p52 subunit, together indicative of constitutive NF-κB2 activation (**Fig. 4A**), which was correctable with re-expression of *TRAF3* (**fig. S7A-D**). The use of BLCLs is a limitation of this study, as EBV immortalization can impact upon NF-κB pathway activation via LMP-1, and the B cell subset from which BLCLs are derived could vary between *TRAF3*^{HI} patients and controls. For discussion of this issue, and results showing that LMP-1 expression and B cell origins are similar between patients and controls, see **fig. S8**.

Further functional studies focused on primary patient B cells. The transcriptomes of sorted naïve and memory B cells from *TRAF3*^{HI} patients showed upregulation of the TNF-NF-κB and CD40 pathway gene sets respectively, consistent with state of increased basal NF-κB2 activation (**Fig. 4B and fig. S7E**). It should be noted that in *TRAF3*^{HI} patients this memory ($CD19^+CD27^+$) population will contain fewer class-switched memory B cells ($CD19^+IgM^-CD27^+$) which may contribute to the observed differences. B cell activation factor (BAFF) is

an important regulator of peripheral B cell survival via NF-κB2 (*22*). Consistent with this, *TRAF3*^{HI} patients' naïve B cells showed heightened NF-κB2 responses to BAFF (**Fig. 4C**). Together these data indicate that the NF-κB2 pathway is hyperactive in *TRAF3*^{HI} patient B cells, and these enhanced responses to BAFF in B cells may be an important factor for the risk of developing autoimmunity, as was evident in BAFF-transgenic mice (*23*).

Despite the increase in NF-κB2, stimulation of *TRAF3*^{HI} patient B cells with anti-IgM/anti-CD40/IL-21 or anti-CD40/IL-21 did not result in convincing differences in plasmablast formation or increased IgG production compared with controls (**fig. S9**), which might be explained by redundancy between TRAF3 and TRAF2 in CD40 signaling (*24*). Recently, it has been reported that TRAF3 is a negative regulator of BCR signaling, with *Traf3*-deficient murine B cells showing elevated intracellular signaling cascades after BCR stimulation (*25, 26*). In *TRAF3*^{HI} patient B cells, stimulation with anti-IgM/IL-4 resulted in increased CD38 and HLA-DR expression (**Fig. 4D**), and in increased phosphorylation of ERK and AKT, both consistent with enhanced BCR signaling (**Fig. 4E**). Furthermore, T*RAF3*^{HI} patient B cells showed upregulated gene expression signatures associated with BCR signaling (**fig. S10**). Therefore, as with *Traf3*-deficient murine B cells, *TRAF3*^{HI} patient B cells appear to have increased BCR responsiveness.

In B cells TRAF3 regulates pro-inflammatory pathways in addition to NF-κB2 and, consistent with this, patients' B cells demonstrated upregulation of 'inflammatory response' and 'JAK – STAT3' gene signatures (**fig. S11A-D**), and showed enhanced STAT3 phosphorylation in response to IL-6, and perhaps to IL-10 at later timepoints (**fig. S11E-H**). The enhanced response to IL-6 may contribute to the hypergammaglobulinemia in *TRAF3*^{HI}

patients. *TRAF3*^{HI} patient B cells also showed upregulation of the mitogen associated protein kinase (MAPK) - activator protein-1 (AP-1) transcription factor gene signatures (**fig. S12A, B**), reinforcing that B cell hyperactivation in human *TRAF3* haploinsufficiency is driven by dysregulation of a number of pro-inflammatory pathways due to the pleiotropic effects of TRAF3 in B cells (**fig. S12C**).

Mitochondrial respiration in *TRAF3* haploinsufficient B cells

B cell activation is associated with increased mitochondrial respiration (*27*), and patient BLCLs showed an increased oxygen consumption rate (OCR), indicative of elevated respiration, which was reduced by TRAF3 re-expression (**Fig. 4F**). Consistent with this increased OCR, both COX II (a component of the mitochondrial electron transport chain complex IV) expression and cytochrome *c* oxidase activity were increased in patients (**Fig. 4G and fig. S13A, B**). This increase in mitochondrial respiration was accompanied by altered mitochondrial morphology, with patient BLCLs showing darkened and thickened mitochondrial cristae by electron microscopy, which normalized following re-expression of wild-type *TRAF3* (**Fig. 4H**). Re-expression of wild-type *TRAF3,* or silencing of *NFKB2,* reduced both these metabolic abnormalities and the expression of the mitochondrial co-transcriptional regulator peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC1α), the master regulator of mitochondrial biogenesis (*28*) (**Fig. 4I, J and fig. S13C**). Thus, reduced TRAF3 leads to an increase in mitochondrial respiration, and demonstrates a link between NF-κB2 and mitochondrial capacity in B cells.

Common variants in *TRAF3* impact on disease risks

We sought to use the relatively discrete clinical features of this *TRAF3*^{HI} syndrome to focus genetic analyses, reducing multiple testing corrections, and allowing us to further define the

impact of common *TRAF3* variants on human disease. We thus analysed associations between *TRAF3* variants and bacterial infections, SLE, Sjögren's syndrome, celiac disease and immunoglobulin levels, also adding B cell malignancy as it occurs in 10% of cases of Sjögren's Syndrome (*29*) and as somatic loss-of-function *TRAF3* variants are frequent in myeloma and diffuse large B cell lymphoma (*30, 31*). Meta-analysis of GWAS identified previously unreported association at the *TRAF3* locus with myeloma and non-Hodgkin's lymphoma (rs753490592-C$(T)_{14}$, $P = 2.02\text{x}10^{-7}$, β -0.098) (**Table S1 and fig. S14A**). Mendelian randomization analysis with whole blood expression quantitative trait loci (eQTL) data showed colocalization with *TRAF3 cis*-eQTLs (top eQTL $P_{SMR} = 1.64\text{x}10^{-2}$, $P_{eQTL} = 1.27\text{x}10^{-142}$**,** $\beta_{eQTL} = 0.21$) that reduce *TRAF3* expression (**Fig. 5A, fig S14B, and Table S2**). Reduced *TRAF3* expression also correlated with increased risk of B cell malignancy and worse patient survival in myeloma ($P = 7.9\text{x}10^{-4}$) (**Fig. 5B**). Meta-analysis of SLE GWAS found association at the *TRAF3* locus (rs12436513-A, $P = 4.79\text{x}10^{-8}$, β = -0.102), with associated variants colocalizing with a *TRAF3 cis*-eQTL in $CD4^+$ T cells (**Table S1 and fig. S14C, D**). Common TRAF3 variants also associated with immunoglobulin levels (rs12887521-A, $P = 7.42\text{x}10^{-160}$, β = 0.061), and consistent with this, the lead variant is a *TRAF3* eQTL in EBV transformed B lymphoblastoid cell lines (BLCLs) (*32*) (**Table S1, Fig. 5C, and fig. S14E**). Therefore, common variants in *TRAF3* are associated with risk of polygenic diseases, and these risk-associated loci convey reduced *TRAF3* expression.

Previously, a single case carrying the heterozygous *TRAF3* missense variant NM_145725.3:c.352C>T:p.R118W (rs143813189-T) was reported in association with herpes simplex encephalitis (HSE) via a dominant-negative effect on protein expression (*9*). This *TRAF3* variant is now recognised to have a prevalence of approximately 1 in 200 to 250 in Europeans (*33–35*). The UK Biobank (*34*) contains 2,383 individuals heterozygous, and 4

homozygous, for *TRAF3* p.R118W, none of whom had documented HSE despite HSV IgG serology showing similar HSV exposure to those without p.R118W (**fig. S15A-D**). However, given the relatively low number of HSE cases in this analysis, in an n+1 model analysis with the 'next' R118W carrier encountered having suffered HSE the results would show that R118W conferred an insignificant but increased odds ratio (OR)= 4.17 [95% CI 0.59 – 30.2] P = 0.16. Thus, whilst heterozygosity for TRAF3 R118W is not a highly penetrant cause of HSE, it cannot be excluded that carrier status may convey a small increased risk. Cell transfection with TRAF3 p.R118W showed reduced TRAF3 expression (**fig. S15E**), and B cells from healthy volunteers heterozygous for TRAF3 p.R118W showed a trend towards reduced TRAF3 expression and an increase in NF-κB2 (**Fig. 5D**), together with reduced marginal zone-like B cells and increased antibody-secreting cells **(Fig. 5E,F).** The majority of the latter were IgM negative, as were those wild-type for TRAF3 p.R118 (**fig. S16**). Whilst not associated with HSE in large cohorts, p.R118W was associated with increased immunoglobulin level (OR = 1.02 $P = 4.57 \times 10^{-4}$), bacterial infection (OR = 1.15 $P = 5.10 \times 10^{-3}$), SLE (OR = 2.46 $P = 7.7 \times 10^{-4}$), and myeloma and non-Hodgkin's lymphoma (OR = 1.91, $P = 2.38 \times 10^{-7}$) (**Table S1 and fig. S15F-K**). Thus a range of common variants at the *TRAF3* locus reduce TRAF3 expression and increase the risk of autoimmune disease and B cell malignancy, suggesting they operate through pathological mechanisms shared with the higher penetrance *TRAF3*^{HI} syndrome.

Somatic loss of *TRAF3* in malignant B cells

Somatic loss of *TRAF3* is a frequent finding in myeloma, with loss-of-function mutations reported in up to ~20% of cases (*36*). We wondered whether the functional impacts observed in germline *TRAF3*^{HI} were also seen after somatic loss of *TRAF3*, providing a malignant cell survival advantage, and corroborating observations made in *TRAF3*^{HI} patient B cells. The

TRAF3-deficient myeloma-derived U266 line shows constitutive NF-κB2 activation, and transduction with either wild-type *TRAF3*, or *NFKB2* silencing, reversed this (**Fig. 6A-C**), while also reducing cell proliferation, increasing cell death, and reducing U266 cell OCR (**Fig. 6D-G**). Furthermore, targeting the NF-κB2 pathway, with either the NIK inhibitor SMI1 or the IKKα/β inhibitor BMS-346641, had similar effects (**Fig. 6H-K**). This increased metabolic respiration in B cells with reduced TRAF3 may further promote B cell malignancies *in vivo*, as it is reported that increased metabolic rates also augment EBV B cell transformation (*37*). Alongside increased OCR in *TRAF3*^{HI} patient BLCLs, increased GLUT1 expression was seen, consistent with the enhanced metabolic rates observed (**fig. S17**). Whilst we cannot draw conclusions on the functional impact of all identified somatic *TRAF3* mutations in B cell malignancies, those that are demonstrated to be loss-of-function (*21*) appear to have effects on B cells that are concordant with those associated with germline *TRAF*^{HI}-causing variants. This in turn suggests that, in human B cell malignancies with somatic loss-of-function *TRAF3* mutations, targeting NIK and the NF-κB2 pathway may present a therapeutic approach.

DISCUSSION

We define the monogenic syndrome of *TRAF3*^{HI} in humans. The predominant findings of hypergammaglobulinemia, lymphadenopathy, splenomegaly and associated histological findings, and autoantibody-associated diseases, pointed to a prominent role for hyperactive B cells in the pathology of human *TRAF3*^{HI}. Reduced TRAF3 in B cells resulted in activation of inflammatory intracellular pathways, including those involving NF-κB2, MAPK and STAT3, and increased mitochondrial respiration. These alterations culminated in immune dysregulation, with predisposition to bacterial infection and autoimmune disease. We demonstrate that common germline disease-associated *TRAF3* variants act in a similar way,

through reducing TRAF3, albeit to a lesser extent than observed in the monogenic disease, explaining how both common and rare germline *TRAF3* variants convey disease risks.

Sjögren's syndrome was the most common autoimmune manifestation in the *TRAF3*^{HI} cohort. Primary Sjögren's syndrome patients are reported to have increased IL-17 (*38*, *39*), yet *TRAF3*^{HI} patients have low IL-17 production, perhaps due to the increased NIK-dependent activation of NF-κB2, a similar mechanism to suppression of T_H17 differentiation by second mitochondrial-derived activator of caspase (SMAC) mimetics (*40*). Sjögren's syndrome is comprised of different endotypes associated with differing underlying disease biology (*41*, *42*), so rather than representing a conflict, it is perhaps not surprising that different clinical or immune defects may occur between Sjögren's syndrome driven by *TRAF*^{HI} compared to 'conventional' polygenic disease. The observation that *TRAF3*^{HI} is associated with both Sjögren's Syndrome and exuberant B cell responses, supports recent evidence for B cell-targeted therapy in primary Sjögren's syndrome e.g. anti-BAFF monoclonal antibodies (*42*).

Although only present in 3/9 *TRAF3*^{HI} patients, allergy and atopic presents a possible association with *TRAF3*^{HI}, given patients demonstrate a relative increase of T_H2 cytokine responses. Moreover, common variation at the *TRAF3* locus are associated with risk of atopy (*8*, *43*, *44*), with top SNP associations at the *TRAF3* locus annotated as *TRAF3* whole blood eQTLs, associated with reduced *TRAF3* gene expression (*44*, *45*). Thus, whilst only present in a minority of the patients here, atopic disease may be an additional clinical feature of *TRAF3*^{HI}.

Phenotyping of the *TRAF3*^{HI} patients has allowed us to delineate the systemic effects of reduced TRAF3, and has highlighted the similarities and differences between a human

TRAF3 haploinsufficient state and cell-specific complete lack of TRAF3 in mouse models. Human *TRAF3*^{HI} most closely recapitulates the B cell-specific knockout mouse model. Whilst whole-body *Traf3*-deficient mice show early-onset runting, loss of peripheral white blood cells and death by ten days of life (*46*), development of mice with cell specific *Traf3* deficiencies allowed dissection of the roles of TRAF3 in different cell types. *Traf3*-deficient B cell mice develop lymphoid hyperplasia, hypergammaglobulinaemia, autoimmune disease and B cell malignancies as they age (*17*), with these *Traf3*-deficient B cells demonstrating enhanced survival, increased TLR responses, and metabolic alterations (*17*, *36*). T cell-specific *Traf3*-deficient mice have shown TRAF3 is required for optimal T cell receptor signaling and T cell immunity (*13*), but also functions as a negative regulator of IL-2 signaling and thereby T_{reg} differentiation, resulting in an increase in T_{reg} numbers, as also observed in *TRAF3*^{HI} patients (*12*). Furthermore, akin to *TRAF3*^{HI} patients, T_{reg}-specific *Traf3*-deficent mice show reduced T_{FR} cells, a concomitant increase in T_{FH} cells, and increased germinal centre formation with enhanced IgG production (*15*). It may appear somewhat incongruous that *TRAF3*^{HI} patients had polyclonal hypergammaglobulinemia with reduced circulating class-switched memory B cells. Antigen-activated B cells can differentiate into either plasma cells or memory cells – and control of each outcome is distinct (*47*, *48*). A reduction of memory B cells with normal/raised immunoglobulins has been reported in other IEIs (*49*), which could be driven by increased plasma cell differentiation of either naïve or memory B cells . The enhanced activity of several signalling pathways in *TRAF3*^{HI} patient B cells, in particular increased BCR signalling, could drive such differentiation to antibody secreting cells *in vivo*. T cell help is also likely to influence this balance, with the dysregulation of T cells in *TRAF3*^{HI} patients, particularly increased cT_{FH} cells and reduced cT_{FR}, likely to contribute to B cell hyperactivity and disease. Thus, the

phenotype of human *TRAF3* haploinsufficiency appears to be predominantly driven by B cell hyperactivity, but with T cell dysregulation also likely to play an important role.

Proportions of marginal zone-like B cells were normal in *TRAF3*^{HI} patients and reduced in TRAF3 p.R118W carriers. These observations are unexpected, as marginal zone B cells were increased in B cell-specific *Traf3*-deficient mice (*17*), and BAFF and NF-κB2 play a role in the development and survival of marginal zone B cells in mice (*50*). One reason for his apparent discrepancy might lie in fundamental differences between the marginal zones of mice and humans. Murine marginal zone B cells are primarily neither isotype-switched nor somatically mutated, while those in humans are thought to be derived from and reflect memory B cells (*51*, *52*). Increased B cell activation resulting from reduced TRAF3 might have distinct impacts on these very different cells, perhaps driving expansion in the more "naive-like" mouse cells, but plasma cell differentiation of the memory-derived human marginal zone cells. Differences in TRAF3 expression may also play a complex role – with TRAF3 expression being partially reduced in many cell types in human *TRAF3*^{HI} and R118W individuals, while its absence is complete but confined to a single cell type in B cell-specific *Traf3*-deficient mice. These conflicting observations underline the importance of studying immunology in humans as well as mouse models.

The increased mitochondrial respiration resulting from reduced TRAF3 was evident in both the BLCLs of *TRAF3*^{HI} patients and the *TRAF3*-deficient plasmacytoid B cell U266 cells with increased OXPHOS. In both cell types re-expression of TRAF3 could reduce OCR. Recently, TRAF3 has been reported to control B cell survival under stress by mobilizing to the mitochondria where it interacts with mitochondrial fission factor, triggering mitochondria-dependent apoptosis (*53*). It was also noted that *TRAF3*-deficient B cells displayed increased

mitochondrial respiration, and larger, more abundant mitochondria (*53*) - similar to our observations in *TRAF3*^{HI} BLCLs. Thus, reduced TRAF may provide further survival advantages to B cells by both reducing apoptotis and increasing mitochondrial respiration, increasing the risk of B cell malignancy.

TRAF3^{HI} revealed a biological link between TRAF3, NF-κB2, and enhanced mitochondrial biogenesis in B cells, driving B cell hyperactivity and thence a syndrome of immune dysregulation, autoimmunity and systemic inflammation. Clinical features of the syndrome enabled a genetic exploration of the impact of *TRAF3* variants across a spectrum of allele frequencies, and demonstrated that more frequent *TRAF3* variants, which reduce *TRAF3* expression, increase the risks of B cell malignancy, infections and autoimmune disease in the general population.

MATERIALS AND METHODS

Study design

This study was designed to investigate adult and pediatric patients with signs and symptoms suggestive of immunodeficiency and/or autoimmunity. All studies had ethical and regulatory committee approvals. All participants or parents/guardians provided written informed consent and assent. Details of patient genetic investigations and *TRAF3* common variant analyses and in the Supplementary Materials.

Cell immunophenotyping

For flow cytometry, PBMCs from patients and controls were stained with fluorochrome conjugated antibodies described in table S5 with gating strategies as in Figures and **fig. S18**. FACS was performed on LSRFortessa X-20 (BD Biosciences) and acquisition was performed with FACS Diva software (BD Bioscience). Further analysis was performed with FlowJo software (LLC). Further details, including conjugated antibodies details, RNAseq, BCR sequencing, western blot, and electron microscopy methods, are described within the Supplementary Materials.

B cell stimulation and proliferation

PBMCs were labelled with CFSE 0.5μM in PBS for 10 minutes at 37^{o}C under constant agitation. PBMCs were corrected for B cell number of 20,000/well and were then cultured in 48-well flat bottom plates for 6 days at 37^{o}C 5% CO_2 in complete RPMI with CpG oligodexocynucleotide 2006 200μg/ml (Invivogen) and human IL-2 50U/ml (R&D Systems), or saturating anti-IgM, anti-CD40, and IL-21 25ng/ml, or anti-CD40 and IL-21 (25ng/ml). B cell proliferation was measured using CFSE dilution and regulation of CD38. PBMCs,

corrected for B cell number, were stimulated with anti-IgM (5ug/ml) and IL-4 (1ug/ml) for either 72 hrs then analysed for CD38 and HLA-DR expression.

Cytokine analysis

PBMCs corrected for T cell number were simulated with anti-CD3/CD28 beads (Miltenyi DynaBeads) for 6 days, then supernatants were analysed IFN-γ, IL-17, IL-4, IL-13 and IL-10 by ELISA. For IL-2 in patient A1, PBMCs corrected for T cell number were stimulated with anti-CD3/CD28 beads (Miltenyi Dynabeads) for 48hrs then supernatant was analysed by ELISA. BLCLs were incubated in complete RPMI with TLR9 ligand ODN2006 5μM (Invivogen). Samples were incubated at 37^{o}C 5% CO_2 for 48 hours then supernatants were removed from cells and analyzed with V-plex Proinflammatory Cytokine Assay (MesoScale Discovery) according to the manufacturer's instructions. Samples were run in triplicate and mean result calculated. For monocyte and dendritic cytokine analysis PBMCs were incubated in complete RPMI with lipopolysaccharide (Invivogen) 500ng/ml for 5hours and supernatants analysed by ELISA.

Cell transduction and transfection

For lentiviral transduction, BLCLs or U266 cells were plated into a 24 well plate in complete RPMI with 5μg/ml polybrene at a cell concentration of 10^6/ml. Lentiviral particles of *TRAF3* (TRAF3 NM_14725 Human Untagged Clone, cat#SC309131, Origene) in a lentiviral construct, or lentiviral particles containing *NFKB2* shRNA (cat# sc-29409-V, Santa Cruz Biotechnologies) or lentiviral particles containing mock shRNA (cat#sc-108080, Santa Cruz Biotechnologies) were added, mixed, and incubated at 37°C for 16 hrs. Cells were then washed and re-plated in complete RPMI at 5x10^5cells/ml and incubated for 48hrs. Following this, puromycin selection was performed in escalating concentrations of puromycin to a

maximum of 2μg/ml until stable colonies were established. Transduction knockdowns or protein re-expressions were checked by western blot for protein expression for every culture. For transfection, BLCLs or U266 cells transfections were performed with Lipofectamine 3000 (Invitrogen, cat#L3000001) according to the manufacturer's instructions. Cells were incubated in Opti-MEM reduced serum media. TRAF3 (NM_14275) Human tagged ORF clone pCMV6-Entry (Origene, cat#RC210417) was altered to remove the Myc-DDK tag by inserting a stop codon after the ORF. For p.R118W the nucleotide at position 352 within the ORF was altered C>T. For Y425X the nucleotide at position 1274 within the ORF was altered C>G. Following transfection cells were incubated in complete RPMI for 48 hours prior to cell lysis. Further methods for B cell EBV immortalisation and U266 cell experiments are described in the Supplementary Materials.

Metabolic assessments

BLCLs were plated in Agilent Seahorse XFe96 Cell Culture microplates at $2x10^5$ cells/well in XF96 RPMI Seahorse media supplemented with glucose 1μM, pyruvate 1μM and L-glutamine 1μM (all Agilent). Cells were then treated sequentially with reagents according to the Mitochondrial Stress Test Kit (Agilent) or the Glycolytic Stress Assay (Agilent) manufacturer's instructions. Oligomycin was used at a final concentration of 1.5μM, carbonyl cyanide-4 (trifluoromethoxy) phenylhydrazone (FCCP) 1μM, rotenone and antimycin 0.5μM, etomoxir (cat# E1905, Sigma) 4μM, and 2-deoxy-d-glucose 50mM. For experiments with cell pre-treatments cells were incubated with either control dimethyl sulfoxide (DMSO) (cat# D8418, Sigma), BMS-345541 10μM (cat# B9935, Sigma) or SMI1 (cat# HY-112433, MedChemExpress) for 16 hours then recounted immediately prior to plating for the assay. BMS-345541 is an Ikkα (IC50 = 4μM) and Ikkβ (IC50 = 0.3μM) inhibitor (*54*). Analysis was performed on a XF96 Seahorse (Agilent). Cytochrome *c* oxidase

activity was assessed with the Complex IV Human Enzyme Activity Microplate Assay Kit (cat# ab109909, Abcam). The assay was performed according to the manufacturer's instructions and all samples were run in triplicate for each of the three biological patient samples and controls. Analysis was performed on a CLARIOstar^{Plus} (BMG Labtech).

Figure Captions

Fig. 1. *TRAF3* haploinsufficiency in patients.

(**A**) Clinical traits present in patients and number (effected/total cohort). Panel created using BioRender. (**B**) Histology (40x magnification) from lobectomy in E1 showing dilated bronchi (bronchiectasis) surrounded by lymphoid follicles (black arrows), and lower panel, dilated bronchi (star) and follicular bronchitis with marked lymphoid hyperplasia forming follicles (black arrows). (**C**) Histology from the spleen of patient B3 with hematoxylin and eosin (HE) staining of numerous large reactive germinal centers (black arrows) (left upper panel), with normal architecture of a single reactive germinal center (right upper panel). Immunohistochemistry of T cell ($CD3^+$ left middle panel) and B cell ($CD19^+$ right middle panel) zonation within the reactive germinal centers and below, IgD staining delineating the germinal center (GC) and marginal zone (MZ). (**D**) Pedigrees of patients with *TRAF3* mutations (Y=tyrosine, S=serine, P=proline, R=arginine, Q=glutamine, X=termination). (**E)** Locations of mutations on a linear TRAF3 cartoon. (**F**) Fold RNA expression of *TRAF3* in peripheral blood mononuclear cells (PBMCs) from patients (n=4) and control (n=5) normalized to *B2M* and *ACTB*. (**G**) Western blots of TRAF3 in patients with unique mutations and controls (Ctrls) from PBMCs, and left**,** bar graph of quantification of TRAF3 protein in patients (n=4) and controls (n=11). No truncated TRAF3 protein products were detected in any patients. Bar graphs show mean ± s.e.m. P values by two-way unpaired t test.

Fig. 2. T cell immunophenotype in *TRAF3*^{HI} patients.

(**A**) Bar graph of total $CD3^+$ T cell and (**B**) $CD4^+$ T cell numbers in *TRAF3*^{HI} patients (Pts, n=9) and controls (Ctrls, n=10). (**C**) Gating of naïve ($CD3^+CD4^+CD45RA^+CCR7^+$), T central memory (TCM, $CD3^+CD4^+CD45RA^-CCR7^+$), T effector memory (TEM, $CD3^+CD4^+CD45RA^-CCR7^-$), and terminally differentiated effector memory RA^+ (TEMRA, $CD3^+CD4^+CD45RA^+CCR7^-$) with bar graph of percentages in Ctrls (n=10) and (n=4). (**D**) Plots of T_{reg} gating ($CD3^+CD4^+CD25^+FOXP3^+$) and bar graph of T_{reg} percentage of $CD4^+$ in Ctrls (n=10) and Pts (n=4). (**E**) Bar graph of $CD8^+$ T cell numbers in controls (n=10) and patients (n=9). (**F**) Gating of naïve, TCM, TEM, and TEMRA $CD8^+$ T cells with bar graph of percentages in Ctrls (n=10) and Pts (n=4). (**G**) Graphs of IFN-γ, IL-17A, TNFα, IL-4, IL-13, and IL-10 production from PBMCs, corrected for T cell number, with anti-CD3/CD28 stimulation in Pts (n=6) and Ctrls (n=8). (**H**) Plots of ($CD3^+CD4^+CXCR5^+CD45RA^-CD62L^-PD1^+$) cT_{FH} in Ctrl and A1 with bar graph of cT_{FH} percentages in Ctrls (n=10) and Pts (n=4). (**I)** Hierarchical clustered gene expression heatmap from sorted cT_{FH} cells of Pts (n=3) and Ctrls (n=4). (**J**) ICOS expression on cT_{FH} in two Ctrls and D1, and (**K**) FACS plots of cT_{FR} ($CD3^+CD4^+CD45RA^-CXCR5^+PD1^+FOXP3^+$). Bar graphs all show mean ± s.e.m. P values by Mann Whitney U test.

Fig. 3. B cell immunophenotyping in *TRAF3*^{HI} patients.

(**A**) Bar graph of B cell numbers ($CD19^+$ cells) in *TRAF3*^{HI} patients (Pts, n=8) and controls (Ctrls, n=10). (**B**) Proportions of marginal zone-like ($CD19^+CD27^+IgM^+$) and class-switched memory B cells ($CD19^+CD27^+IgM^-$)in Pts (n=7) and Ctrls (n=10). P values calculated by Mann Whitney U test. (**C**) Plots of the proportions of IgM and class-switched memory IgG^+, IgA^+ B cells in a Ctrl and Pts. (**D**) Flow cytometry plots of $CD19^+$ $CD21^{low}CD38^-Tbet^+$ B cells, and graph of percentages in Pts (n=6) and Ctrls (n=10). (**E**) Plots of antibody secreting cells (ASCs) ($CD19+CD27^{++}CD38^{++}$) from Ctrl and C2, and bar graph of the ASCs percentage of total $CD19^+$ B cells in Ctrls (n=10) and Pts (n=7). P values calculated by Mann Whitney U test. (**F**) FACS plots of B cell proliferation and plasmablast differentiation a Ctrl and in C2 by $CD38^+$ upregulation and CFSE dilution from culture of PBMCs, corrected for B cell number, unstimulated or stimulated with CpG (200ug/ml) and IL-2 (50 U/ml) for 6 days. Graph of the percentages of $CFSE^{low}$ $CD38^+$ B cells in Ctrls (n=10) and Pts (n=6) after 6 days of stimulation of PBMCs. Graphs show mean ± s.e.m. P value by two-way unpaired t test.

Fig. 4. Enhanced inflammation and metabolism in patient B cells.

(**A**) Western blots of NIK, phosphorylated NF-κB2 p100 (p-p100), NF-κB2 p100 and p52 from whole cell BLCL lysates from controls (Ctrls) and *TRAF3*^{HI} patients (Pts), and bar graphs of quantifications of western blots from patients (n=3) and controls (n=9). Bar graphs show mean ± s.e.m. *P* values by two-way unpaired t test. (**B**) Gene set enrichment of the TNF – NFKB pathway in Pts' naïve B cells ($CD19^+CD27^-$). (**C**) Flow histograms of intranuclear NF-κB2 in Ctrls (n=4) and Pts (n=2) gated on naïve B cells ($CD19^+CD27^-$) from PBMCs unstimulated or with BAFF 100ng/ml for 24 hrs. (**D**) Histograms of CD38 and HLA-DR expression on B cells ($CD19^+$) from PBMCs, corrected for B cell number, stimulated with anti-IgM (5ug/ml) and IL-4 (50ng/ml) for 72 hrs. (**E**) Histograms of phosphorylated ERK and AKT in $CD19^+$ B cells from PBMCs stimulated with anti-IgM for 7 minutes. (**F**) OCR of Ctrl BLCLs (n=3) with empty vector transduction (EV), Pt BLCLs (n=3) with EV, and *TRAF3*$^+$ wild-type transduced Pt BLCLs (n=3) with sequential administration of oligomycin (Oligo), carbonyl cyanide-p-trifluoromethoxy phenylhydrazone (FCCP), and rotenone/antimycin A (Rot/AA). Line graph shows mean ± s.e.m. 3 biological samples each with 5 replicates. (**G**) Activity fluorescence decay curve of assessment of cytochrome *c* oxidase activity from mitochondria of A1 and Ctrl BLCLs, with bar graph of cytochrome *c* oxidase activity normalized against controls for Ctrls (n=3) and Pts (n=3) BLCLs. Bar chart shows mean ± s.e.m. P value calculated by two-way unpaired t test. (**H**) Transmission electron microscopy images of mitochondria morphology in EV transduced control BLCLs (Ctrl EV), EV transduced *TRAF*$^{HI-}$ BLCLs from B1, and B1 BLCLs transduced with wild type *TRAF3*$^+$ expressing vector. Images representative of 2 experiments. (**I**) Western blot of NF-κB2 p100 and p52, and PGC1α in control BLCLs transduced with either mock short hairpin (shRNA) or *NFKB2* shRNA. (**J**) OCR in the mock shRNA or *NFKB2* shRNA transduced BLCLs

treated with oligo, FCCP and Rot/AA at timepoints as indicated. Line graph of mean ± s.e.m. from 5 replicates.

Fig. 5. Impacts of common variation at the *TRAF3* locus associated with immune diseases. (**A**) Inverse correlation of whole-blood *TRAF3* eQTL effect size on *TRAF3* expression and effect size from myeloma and non-Hodgkin's lymphoma GWAS meta-analysis. (**B**) Kaplan-Meier survival curves for myeloma patients with high or low *TRAF3* expression in myeloma cells, and time (days) from diagnosis. (**C**) Funnel plots of the effect of rs12887521 eQTL zygosity on *TRAF3* expression in BLCLs from GTEx v8 (*32*). (**D**) Western blot and bar graphs of quantifications of TRAF3 and NF-κB2 p100 and p52 subunits in sorted B cells ($CD19^{+}$) from healthy volunteers wild-type for TRAF3 p.R118 (Ctrls, n=5) and healthy volunteers heterozygous for TRAF3 p.R118W (n=5). Bar graphs show mean ± s.e.m. *P* values for TRAF3 and p52 by two-sided t test, *P* value for p100 by Mann-Whitney U test. (**E**) Flow cytometry plots of CD27 and IgM expression on B cells in a TRAF3 p.R118 wild-type (Ctrl) and p.R118W carrier. (**F**) Flow cytometry plots of CD27 and CD38 on B cells each with bar graphs of controls (n=5) and p.R118W carriers (n=5). MZ-like, marginal zone-like ($CD19^{+}CD27^{-}IgM^{+}$); ASC, antibody secreting cells ($CD19^{+}CD27^{++}CD38^{++}$). Bar graphs show mean ± s.e.m. *P* values calculated by two-sided unpaired t test.

Fig. 6. Impacts of somatic loss of *TRAF3*. (**A**) Sanger sequencing fluorescent traces from a control BLCL line and U266 cells showing insertion of a deoxythymidine (red arrow) leading to a stop-codon. (**B**) Bar graph of relative gene expression of *TRAF3,* normalized to wild-type *TRAF3* from control BLCLs, in U266 cells transduced with an empty vector (EV), *NFKB2* shRNA (*NFKB2* KD), or wild-type *TRAF3* (TRAF3+). (**C**) Western blot of TRAF3, NF-κB2 p100 and p52 in U266 cells transduced with either mock shRNA, *NFKB2* shRNA, or wild-type *TRAF3*$^{+}$ expressing vector. (**D**) Line graph of U266 cell death percentages, (**E**) U266 cell proliferation, (**F**) U266 cell numbers over time from selected stably transduced colonies, and (**G**) OCR measurement of U266 cells transduced with either mock shRNA, *NFKB2* shRNA or wild-type *TRAF3*$^{+}$ over time with administration of oligomycin (Oligo), carbonyl cyanide 4-trifluoromethoxy phenylhydrazone (FCCP), and rotenone and antimycin A (Rot/AA). Representative of two independent experiments with 3 replicates each. Line graphs show means ± s.e.m. (**H**) U226 percentage cell death, (**I**) U266 cell proliferation, and (**J**) total U266 cell numbers over time when treated with Ctrl (DMSO), or NIK inhibitor SMI1 10μM, IKKα/β inhibitor BMS-345541 10μM, or bortezomib 5nM. (**K**) OCR measurement of U266 cells pre-treated for 6 hours with SMI1, BMS-345541, or bortezomib. Representative of two independent experiments. Line graphs shows means ± s.e.m. from 3-5 replicates for each condition and timepoint.

Table 1. Clinical phenotypes of patients

	Family A	Family B				Family C		Family D	Family E
Patient	A1	B1	B2	B3	B4	C1	C2	D1	E1
Age, gender	40, M	10, F	15, M	47, F	71, F	15, F	50, M	15, M	20, M
Bacterial Infections	Recurrent sinopulmonary infections, Recurrent otitis media	Recurrent sinopulmonary infections, Recurrent otitis media Lymphadenitis, Pulmonary NTM	Recurrent otitis media, Recurrent sinusitis	Recurrent sinopulmonary infections, Lymphadenitis Peritonitis	Recurrent sinopulmonary infections	Recurrent sinopulmonary infections, Lymphadenitis	Recurrent sinopulmonary infections	Recurrent otitis media	Recurrent otitis media, Recurrent sinopulmonary infections, Pulmonary NTM
Lymphadenopathy	Splenomegaly GI lymphoid hyperplasia Pulmonary	Pulmonary, Splenomegaly	UNK	Splenomegaly	UNK	Pulmonary	UNK	Tonsils, Adenoids, Peripheral	Cervical, Inguinal, Pulmonary Splenomegaly
Respiratory	Bronchiectasis	Bronchiectasis	-	Bronchiectasis	Bronchiectasis	Bronchiectasis	-	-	Bronchiectasis, Follicular bronchitis.
Immune dysregulation	Enteropathy, Sicca syndrome	-	-	Sjögren's syndrome	Sjögren's syndrome, Vasculitis	Celiac disease	Atopic dermatitis, Allergic sensitization	Hashimoto's thyroiditis, Systemic juvenile inflammatory arthritis Food and drug allergy, Atopic dermatitis Allergic sensitization	Vasculitis, Antiphospholipid syndrome, Atopic dermatitis
Immunoglobulins (g/L)	IgG 9.4*↔ IgA <0.01*↓ IgM <0.2*↓ IgE <2 IU/ml Polysaccharide responses ↓ Tetanus abs 0.21 IU/ml	IgG 13.5↑ IgA 1.6↔ IgM 2.3↔ IgE 6.5IU/ml Polysaccharide responses ↓ Tetanus abs 42.5 IU/ml	IgG 18.2↑ IgA 3.7↔ IgM 2.8↑ Polysaccharide responses ↓	IgG 19.5↑ IgA 2.8↔ IgM 6.3↑ Polysaccharide responses UNK	IgG 11.4↔ IgA 1.6↔ IgM 2.5↔ Polysaccharide responses UNK	IgG 17.3↑ IgA <0.01↓ IgM 1.5↑ IgE 116IU/ml Polysaccharide responses ↓ Tetanus abs 4.7 IU/ml	IgG 16.9↑ IgA 4.5↑ IgM 1.8↔ Polysaccharide responses UNK	IgG 19.5↑ IgA 4.8↑ IgM 2.0↑ IgE 2477 IU/ml↑ Polysaccharide responses UNK Tetanus abs 0.8 IU/ml	IgG 31.6↑ IgA 0.44 IgM 0.82 IgE 2.07 IU/ml Polysaccharide responses ↓
Autoantibodies	Anti-Ro	-	-	Anti-Ro, Anti-La	Anti-Ro, Anti-La, ANCA PR3	TTG IgG	UNK	Anti-TPO, Anti-Tg, Thyroid stimulating antibodies	Anticardiolipin, B_2 glycoprotein, Lupus anticoagulant, ANCA MPO / PR3

Clinical and immunological phenotypes of the patients. Immunoglobulin level normal ranges based on clinical laboratory age adjusted values. ↔ = normal, ↑ = raised, ↓ = low. NTM = non-tuberculous mycobacteria, ANCA = anti-neutrophil cytoplasmic antibodies, TTG = tissue transglutaminase, TPO = thyroid peroxidase, Tg = thyroglobulin, UNK = unknown, - = not present, * = on immunoglobulin replacement.

Supplementary Materials

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