Interferon autoantibodies associated with AIRE deficiency decrease the expression of IFN-stimulated genes

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Neutralizing autoantibodies to type I, but not type II, interferons (IFNs) are found at high titers in almost every patient with autoimmune polyendocrinopathy candidiasis ectodermal dysplasia (APECED), a disease caused by AIRE gene mutations that lead to defects in thymic T-cell selection. Combining genome-wide expression array with real time RT-PCR assays, we here demonstrate that antibodies against IFN-α cause highly significant down-regulation of interferon-stimulated gene expression in cells from APECED patients’ blood by blocking their highly dilute endogenous IFNs. This down-regulation was lost progressively as these APECED cells matured in cultures without neutralizing autoantibodies. Most interestingly, a rare APECED patient with autoantibodies to IFN-α but not IFN-α showed a marked increase in expression of the same interferon-stimulated genes. We also report unexpected increases in serum CXCL10 levels in APECED. Our results argue that the breakdown of tolerance to IFNs in AIRE deficiency is associated with impaired responses to them in thymus, and highlight APECED as another autoimmune disease with associated dysregulation of IFN activity. (Blood. 2008;112:2657-2666)

Introduction

Type I interferons (IFNs) are cytokines with pleiotropic activities that contribute to early defense against pathogens, development of adaptive immunity, and protective antitumor responses. The human type I IFN gene family consists of 13 distinct functional IFN-α, and single IFN-β, IFN-ε, IFN-κ, and IFN-ω genes; the respective IFN molecules all use the same cell surface receptor complex, IFN-α receptor.1,2 Although seminal studies reported the expression of type I IFNs by monocytes,3 IFN-α, -β, and -ω are secreted in much larger amounts by dendritic cells (DCs), above all by plasmacytoid DCs.4,5 However, virtually all nucleated cells can produce some type I IFNs after viral infection. The activation of IFN genes in DCs depends on IFN regulatory factors 7 (IRF7) and 3 (IRF3), the former termed “master regulator of type I IFN synthesis.”6 After secretion and receptor binding, membrane-proximal immediate signaling is initiated through the catalytic activation of receptor-associated JAK1 and TYK2 tyrosine kinases. Transcription factors in the signal transducer and activator of transcription family members (STAT1 and STAT2) are then attached to the activated receptor complex via phosphoryrosine recruitment motifs and then undergo phosphorylation on tyrosine and, in complex with IRF9 protein, are translocated to the nucleus to up-regulate the expression of IFN-stimulated genes (ISGs).1 Type I IFNs are deeply implicated in pathogenesis of certain autoimmune diseases. In particular, in the chronic systemic autoimmune disease, systemic lupus erythematosus (SLE),2,5,7,9 IFN-α serum levels are elevated in patients with severe SLE and associated with the frequent up-regulation of ISGs, the so-called IFN signature in their peripheral blood mononuclear cells (PBMCs).

Recently, we reported high titer neutralizing autoantibodies to type I, but not type II, IFNs in autoimmune polyendocrinopathy candidiasis ectodermal dysplasia (APECED or APS1) patients,10 a recessive disorder resulting from mutations in the autoimmune regulator (AIRE) gene (7). Although AIRE has been detected in medullary thymic epithelial cells (mTECs) where it is thought to control autoimmunity by regulating the expression of peripheral tissue-restricted antigens that induce self-tolerance in developing T cells,11,16 Most APECED patients develop multiple endocrine
autoimmune diseases, often with high levels of serum autoantibodies against components of the affected organs. Highly variable clinically, APECED usually begins in infancy with chronic Candida infection, followed by autoimmune attack on the parathyroids, adrenal cortex, and/or gonads, endocrine cells in the gut, pancreatic islets, thyroid gland, and others.17 The prevalence of organ-specific autoantibodies in APECED patients varies between 8% and 66%.18 For those against IFN-α or IFN-γ, it reaches 100% or more than 95%, respectively.10,18 However, anti-IFN antibodies have not been reported in Aire-deficient mice, which do not precisely reproduce many characteristics of APECED, notably the candidiasis.20 Similar anti-IFN autoantibodies are also found in many patients with thymoma, especially those with myasthenia gravis.21

Here we present new evidence supporting our hypothesis that, by specifically neutralizing IFN-α, these autoantibodies decrease expression of ISGs in APECED peripheral blood cells. The down-regulation of ISGs was not caused by any inherent defect in the patients’ monocyte-derived DCs or plasmacytoid DCs, as it was reversible if they were cultured without neutralizing autoantibodies. We also report increase in serum chemokine (C-X-C motif) ligand 10 (CXCL10; alias IP-10) levels in APECED that most probably reflect increased production of proinflammatory cytokines in the target tissues.

Methods

Patients

With local approval from the Ethics Review Committee on Human Research of the University of Tartu, Estonia, and informed consent in accordance with the Declaration of Helsinki, we studied 8 APECED patients and age-matched controls. Their ages at sample collection, APECED features, and organ-specific autoantibodies and AIRE genotypes are given in Table 1. They all had high titers of neutralizing autoantibodies against IFN-α, and the majority against the IFN-α in addition (Table 2), but patient A3 proved to be negative and patient A2 weakly positive in antiviral neutralization assays.10 None of the patients was taking systemic immunosuppressive treatment at the time of the sampling. Sera from Norwegian APECED and Addison disease collection, the Finnish APECED collection, a Sardinian APECED and unaffected heterozygous relative cohort, and some U.S. APECED patient sera and SLE sera from Tartu University Clinic serum bank were used for cytokine measurements.

Cell isolation, dendritic cell generation, and cell stimulation

PBMCs were separated from heparinized blood samples on Ficoll-Paque PLUS (GE Healthcare, Little Chalfont, United Kingdom). Table 1. Disease characteristics, autoantibodies, and AIRE mutations of APECED patients of this study

| Patient no. | Sex | Age, y | Autoantibodies | Manifestations | AIRE mutations |
|------------|-----|--------|----------------|---------------|----------------|
| A1         | F   | 54     | 21OH, SCC, AADC, GAD | C, HP, A, V, AI, | c.769C>T/c.769C>T |
| A2         | M   | 47     | AADC, GAD, TPH | HP, D, V | c.879G>A/c.879G>A |
| A3         | F   | 54     | 21OH, GAD | C, A, HP, G, AT, E, D | c.769C>T/c.1336T>G |
| A4         | F   | 25     | 21OH, 17OH | A | c.769C>T/c.1336T>G |
| A5         | F   | 20     | 21OH, SCC, 17OH | C, A, V, N, G | c.769C>T/c.1242_1243insA |
| A6         | M   | 21     | 21OH, SCC, 17OH, AADC | C, HP, A, K | c.769C>T/c.769C>T |
| A7         | M   | 17     | negative | C, HP, MT | c.1064-1066dupCCCCGG/c.1064-1066dupCCCCGG |
| A8         | F   | 56     | 21OH, 17OH | C, HP, A | R257X/97-97del13 |

21OH indicates 21-hydroxylase; SCC, side-chain cleavage enzyme; AADC, aromatic L-amino acid decarboxylase; GAD, glutamic acid decarboxylase; TPH, tryptophan hydroxylase; 17OH, 17α-hydroxylase; A, adrenal insufficiency; HP, hypoparathyroidism; C, mucocutaneous candidiasis; G, primary gonadal insufficiency; V, vitiligo; AI, alopecia; AT, autoimmune thyroid disease; M, malabsorption; N, nail pitting; E, dental enamel hypoplasia; K, keratopathy; and D, type I diabetes.

Table 2. The titers of anti-IFN neutralizing autoantibodies in APECED patients of this study

| Patient no. | Neutralizing titer |
|-------------|--------------------|
|             | IFN-α 2 | IFN-α 8 | IFN-α50 | IFN-α50 |
| A1          | 90 000  | 90 000  | 50 000  | < 40     |
| A2          | 100     | 300     | 30 000  | < 40     |
| A3          | 60      | 40      | 22 500  | < 40     |
| A4          | 110 000 | 256 000 | 90 000  | < 40     |
| A5          | 64 000  | 64 000  | 50 000  | < 40     |
| A6          | > 256 000 | > 256 000 | 256 000 | < 40     |
| A7          | > 256 000 | 180 000  | 20 000  | < 40     |
| A8          | 500 000 | 500 000 | 120 000 | 550      |

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for 30 minutes on ice. The cells were stained with 5 μL anti–phospho-STAT1 (Y701)-Alexa Fluor 488 at room temperature for 1 hour, washed, and analyzed using FACScalibur. The mean Alexa Fluor 488-fluorescence intensity (MFI) of the cells was analyzed using CELLQuest software (everything from BD Biosciences). To detect serum IFN-α activity, the cells were incubated in 50% serum for 15 minutes. In some tubes, blocking IFN-α antibody at 10 μg/mL (clone 9D3, Abcam, Cambridge, United Kingdom) or isotype control antibody (BD Biosciences) was added. Data were expressed as MFI units (MFI units = MFIa − MFIb) where MFIa represents value for test sample and MFIb value for background staining.

Statistical analysis

Statistical analysis was done with the help of GraphPad Prism software (GraphPad Software, San Diego, CA). The mean values of different groups were compared using t test in the case of gene expression data and Mann-Whitney test in the case of cytokine concentrations. Bonferroni correction was used for multiple comparisons.

Results

Interferon-regulated gene expression is decreased in APECED blood cells

High-titer anti–type I IFN autoantibodies are found in nearly 100% of AIRE-mutant APECED patients.2,10,19,25 They neutralize the antiviral actions of most type I IFNs, especially IFN-α, but IFN-β less frequently and the type II IFN-γ almost never. To assess their potential effects on ISG expression patterns, we performed Affymetrix Human Genome U133 Plus 2.0 Array analyses on monocytes and monocyte-derived DCs. Readily isolated in large numbers from APECED and control subjects’ blood, these cells are known type I IFN producers and responders. After normalization, the genes showing more than 1.5-fold changes in each of 2 experiments were considered to be differentially expressed. Using literature surveys, we selected a total of 285 genes (Table S2) that are up-regulated by IFNs.26,27 Strikingly, 61 of these genes were down-regulated for at least 1.5-fold (Figure 1A), and the number of down-regulated ISGs was highly significantly changed in APECED CD14+ monocytes compared with healthy controls (P = 3.9 × 10−125; Figure 1B). Notably, however, the differences (and their significances) waned as the monocytes matured in culture into immature dendritic cells (MFIa; P = 5.7 × 10−5; Figure 1B) and mDCs (P = 1.9 × 10−2; Figure 1B) as visualized in Figure 1A.

To confirm this ISG down-regulation independently in more APECED patients, we next analyzed the expression of some well-known ISGs that showed reduced expression in our array experiments, using RT-PCR to quantitate transcripts in PBMCs freshly isolated ex vivo. The down-regulation clearly correlated with the presence of neutralizing autoantibodies specific for the IFN-α because it was evident in all the APECED patients with high titers of autoantibodies against several IFN-α subtypes (Figure 2A). In contrast, expression of ISGs was strongly increased in one Norwegian APECED patient with neutralizing autoantibodies against IFN-α only (patient A3; Table 2; Figure 2A). Furthermore, patient A2 with low titers of anti–IFN-α antibodies showed the highest ISG expression levels among APECED patients with neutralizing autoantibodies against IFN-α for 7 of the 8 ISGs tested (data not shown).

To identify the cell types affected, we then analyzed freshly isolated monocytes and plasmacytoid DCs. Notably, both cell types also showed similar down-regulation of the same ISGs (Figure
Moreover, the down-regulated ISG pattern had normalized in monocyte-derived DCs that had differentiated for 6 days in culture with fetal calf serum (Figures 1A, B, 2D). In contrast with the ISGs, we saw no differences between the groups in the expression patterns of genes that are selectively (CIITA) or preferentially (CXCL9) regulated by IFN-β; nor for CCL5, a chemokine induced more by proinflammatory cytokines than type I IFNs, or IFNAR1 (Figure 2E).

Taken together, these results show clearly decreased expression of all the classic ISGs tested in all the APECED patients with neutralizing antibodies against IFN-β, whereas, in one patient who has only anti–IFN-β autoantibodies, it appeared to be strongly enhanced.

**APECED sera with anti–IFN-β inhibit ISG expression**

We next tested for acute effects of the neutralizing autoantibodies after incubating monocytes from healthy donors in medium containing 20% autologous plasma and 2% APECED or control sera for 18 hours. Expression of all the ISGs tested was significantly down-regulated by all the APECED sera that contained anti–IFN-β neutralizing autoantibodies, but not by the one specific for IFN-β (Figure 3A). If fetal calf serum was used instead of autologous serum, no differences in ISG expression were seen in monocytes cultured in the presence of APECED or control sera (data not shown). Evidently, human plasma contains low levels of type I IFNs that can be blocked by the patients’ neutralizing autoantibodies.

To test whether these anti–IFN-β autoantibodies also inhibit IFN signaling, we assessed phosphorylation of STAT1 protein, a crucial early event after stimulation of the IFN receptor complex. Cells treated with IFN-β showed significant phosphorylation of STAT1 protein that was again inhibited, indeed, down to baseline levels, but only by APECED sera containing high titers of neutralizing autoantibodies to IFN-β (Figure 3B). By contrast, the
IFN-α–specific serum A3, and another from one SLE patient, induced STAT1 phosphorylation; that induction was specifically neutralized by anti–IFN-α antibody (Figure 3C). Together, the down-regulation of ISG expression and of STAT1 phosphorylation in the presence of APECED sera shows that the changes observed in the APECED cells are the result of autoantibody neutralization of IFN-α.

APECED patients’ plasmacytoid DCs and monocyte-derived DCs express normal levels of type I IFNs

In response to viral stimulation, plasmacytoid DCs produce 10- to 100-fold more type I IFNs than monocytes. When PBMCs were stained for BDCA-2, a C-type lectin receptor that is specific for plasmacytoid DCs, their frequencies were slightly lower in APECED...
patients than in healthy controls, but not significantly ($P = .079$, Figure 4A).

To assess the potential of these plasmacytoid DCs to autoimmunize against IFNs in APECED, we next measured their IRF and IFN expression after short-term stimulation with influenza virus or CpG. We found no significant differences in transcript levels of their IFN-α2, IFN-α8, or IFN-β (Figure 4B) or of interferon-regulatory factors IRF3 and IRF7 (Figure 4C): indeed, the levels were even slightly lower in APECED than control plasmacytoid DCs. Because myeloid DCs also contribute significantly to the overall production of type I IFNs, we stimulated (precultured) monocyte-derived DCs for 18 hours with poly(I:C). Again, however,
no differences were seen in expression of these type I IFN genes (Figure 3D).

In conclusion, the numbers of plasmacytoid DCs and levels of type I IFN mRNA expression by dendritic cells were comparable in APECED patients and healthy controls, which argues against any inherent hyperactivation in circulating APECED DCs that could lead to the breakdown of tolerance to type IFNs.

**Elevation of serum CXCL10 levels in APECED patients**

There are several recent reports of elevated levels of the proinflammatory chemokine CXCL10 in endocrine autoimmune diseases. Our studies here indicate that the neutralizing autoantibodies to IFN-α decrease the expression of CXCL10 in blood cells from APECED patients (Figure 2A-C). This prompted us to measure their serum CXCL10 levels; they were assessed by quantitative immunoassay in serum samples available from 49 APECED patients, 39 unaffected AIRE heterozygous relatives, and 9 healthy controls. In contrast with the decreased mRNA levels in blood cells, the APECED patients had significantly higher serum CXCL10 protein levels than the unaffected relatives (Figure 5, P < .001). Levels were even higher than in patients with isolated Addison’s disease or autoimmune polyendocrine syndrome type 2 (APS2; P < .01 and P < .05, respectively). In the APECED patients, the CXCL10 levels did not correlate with the exact AIRE mutations, disease-onset ages, or numbers or types of clinical manifestations. Although the values spanned a wide range in each group, nearly all APECED patients were above the medians of the controls or relatives but did vary substantially in serial samples from the same individual Norwegian patients (data not shown).

We next investigated serum levels of proinflammatory cytokines in APECED patients and healthy controls. As with CXCL10, we observed a tendency to higher levels of IL-6 (P = .053) and TNF-α (P < .05) in APECED than in healthy controls (Table S3). However, the levels of IFN-γ, IL-10, IL-5, IL-4, and IL-2 showed no significant changes (Table S3 and data not shown).

**Discussion**

Our experiments clearly demonstrate down-regulated ISG expression in APECED patients’ PBMCs. In sharp contrast, the expression of...
ISGs is higher in SLE patients, which is consistent with their well-known elevations in serum IFN-α levels.\textsuperscript{2,5,7-9,31} The lower expression of ISGs in APECED patients is apparently caused by neutralizing autoantibodies to the IFN-α rather than IFN-β or IFN-ω. Moreover, anti–IFN-α antibody–containing sera clearly down-regulated ISG expression in control monocytes ex vivo and blocked the key IFN-induced early signaling event, STAT1 phosphorylation. Importantly, these effects were reversible; after maturation in fetal calf serum (instead of APECED plasma), these patients’ monocyte-derived DCs showed normal expression of ISGs. We propose that deprivation of the normal low levels of circulating IFN\textsubscript{α} that would normally neutralize IFNs in the bloodstream, they may not reach sufficient levels to do so in the tissues, which might help explain the surprising rarity of viral infections in APECED patients despite their decreased expression of ISGs in blood cells.\textsuperscript{35} Modest increases in CXCL10 have been described in other organ-specific diseases, such as Hashimoto thyroiditis,\textsuperscript{29} Graves disease,\textsuperscript{29} myasthenia gravis,\textsuperscript{36} and Addison disease,\textsuperscript{30} and also during type I IFN treatment.\textsuperscript{37,38} In APECED, the autoimmune processes in endocrine tissues are most probably accompanied by IFN-γ production that is a strong inducer of CXCL10. We propose that chemokines measured in serum are derived not from blood cells but rather from the extravascular target tissues, where they are responding to inflammatory stimuli and thus chemotactically attract immune cells out of the circulation. That might explain the slightly decreased percentages of plasmacytoid DCs in APECED blood (Figure 4A), which have also been noted in SLE.\textsuperscript{39}

Type I IFNs are produced in large quantities during many acute viral infections. In physiologic situations, their actions may largely be localized to infected tissues and operate mainly via autocrine and paracrine stimulation. Although the autoantibodies can effectively neutralize IFNs in the bloodstream, they may not reach sufficient levels to do so in the tissues, which might help explain the surprising rarity of viral infections in APECED patients despite their decreased expression of ISGs in blood cells. In addition, there may be compensation by IFN-β and/or IFN-λ, which are neutralized much less in most patients.

The consistently increased expression of ISGs in APECED patient A3, whose neutralizing autoantibodies only recognize IFN-ω (and not IFN-α), also hints at an underlying local overproduction of type I IFNs in APECED patients who are masked by the anti–IFN-α autoantibodies in the typical cases. Most ISGs are up-regulated by both type I IFNs and IFN-γ. The normal expression of CIITA, which is selectively up-regulated by IFN-γ, argues that it does not contribute substantially and thus implicates the IFN-α most strongly. The CIITA level was even lower in patient A3, and her serum did contain IFN-ω because it evoked significant STAT1 phosphorylation in control cells and could be neutralized by anti–IFN-α antibodies. Notably, one side effect of treatment with IFN-α is thyroid autoimmunity.\textsuperscript{40} Interestingly, the one patient with detectable serum IFN-α and with neutralizing autoantibodies only against IFN-ω (patient A3) also has thyroiditis, which is otherwise rare among Norwegian APECED patients. Similarly, the other serologically similar patient (A2) had thyroid peroxidase autoantibodies.

After virus- or TLR-stimulation, plasmacytoid DCs produce a mixture of type I IFNs, but predominantly IFN-ω, whereas, after TLR 4 or TLR 3 stimulation, monocyte-derived DCs secrete mainly IFN-β,\textsuperscript{41} which is recognized infrequently (~20%) by the neutralizing autoantibodies in APECED.\textsuperscript{10} Moreover, the type I IFNs show distinct actions; the chemokine-stimulating and DC-maturing properties of IFN-ω seem particularly restricted.\textsuperscript{42} This is consistent with the observed ISG up-regulation by the APECED sera with neutralizing autoantibodies against only IFN-ω, in contrast to ISG down-regulation by the APECED sera with autoantibodies to IFN-α (and IFN-ω). Although our results do not indicate any abnormal IFN overproduction in APECED peripheral DCs, the human thymus contains not only numerous DCs but also many plasmacytoid DCs, which can produce large amounts of type I IFNs. Moreover, they are predominantly located in the medulla and corticomedullary junction,\textsuperscript{43} where type I IFNs appear to be constitutively secreted.\textsuperscript{10} Type I IFN overproduction by thymic plasmacytoid DCs impairs Thymic T-cell development.\textsuperscript{44} It is also involved in terminal differentiation and subsequent apoptosis of thymic epithelial cells,\textsuperscript{45} and so, interestingly, is wild-type AIRE.\textsuperscript{46} The coincidence of highly prevalent, high-titer IFN autoantibodies in patients with thymic epithelial tumors or genetic defects in AIRE suggests parallel mechanisms of autoimmunization in the APECED thymus and thymomas. Indeed, the neoplastic epithelial cells in thymomas almost always fail to express AIRE.\textsuperscript{47} We propose that aberrant cell death or some other so far unknown danger signal renders the APECED thymic environment prone to autoimmunize responsive T cells against locally abundant type I IFNs (Figure 6).
In conclusion, we show that ISGs are down-regulated in APECED patients’ PBMCs by their autoantibodies against type I IFNs and demonstrate marked increase in serum CXCL10 levels in APECED. Our results suggest that AIRE deficiency causes increased production of IFNs, possibly through aberrant cell death in the APECED thymus, which results in highly specific autoantibodies to type I IFNs and down-regulation of ISGs in APECED blood cells.

Acknowledgments

The authors thank the patients and controls for so kindly providing the generous samples on which this study crucially depends; Dr Kylie E. Webster and Dr Ken Simpson for help in sample preparation and analysis, respectively, of the Affymetrix chips; Ulla Kiiskinen and Elisabeth Halvorsen for technical assistance; and Jim Robertson for influenza virus. This work was supported by the Wellcome Trust Senior Fellowship grant, EU Framework program 6 (Thymaide and Euraps; P.P.), the European Regional Fund and Archimedes Foundation, the Estonian Science Foundation (grants 6663, 6514 and 7197; K.K., P.P., M.L.), Slovenian Agency for Research (J3-9663; K.T.P. and T.B.), National Health and Medical Research Council (NHMRC) fellowships (171601 and 461204), and National Health and Medical Research Council (NHMRC) program grants (257501, 264573 and 406700; H.S.S.).

Authorship

Contribution: K.K., M.L., A.S.B.W., A. Meager, L.T., A. Murumägi, and H.S.S. performed experiments and analyzed data; K.K. and T.O. performed statistical analysis; A.S.B.W., E.S.H., K.L., K.T.P., T.B., A.L., O.K., A. Meloni, B.E.-L., N.K.M., J.P., K.J.E.K., and R.U. were responsible for collection of clinical material and data; and K.K., N.W., and P.P. designed the research and wrote the paper.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

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References

1. Pestka S, Krause CD, Walter MR. Interferons, interferon-like cytokines, and their receptors. Immunol Rev. 2004;202:8-32.
2. Baccala R, Kono DH, Theofilopoulos AN. Interferons as pathogenic effectors in autoimmunity. Immunol Rev. 2005;204:9-26.
3. Sakseha E, Virtanen I, Hovi T, Secher DS, Cantell K. Monocyte is the main producer of human leukocyte alpha interferons following Sendai virus induction. Prog Med Virol. 1984;30:78-86.
4. Liu YJPC, professional type 1 interferon-producing cells and plasmacytoid dendritic cell precursors. Annu Rev Immunol. 2005;23:275-306.
5. Bianco P, Paluca AK, Pascual V, Banchereau J. Dendritic cells and cytokines in human inflammatory and autoimmune diseases. Cytokine Growth Factor Rev. 2008;19:41-52.
6. Honda K, Yanai H, Negishi H, et al. IRF-7 is the master regulator of type-I interferon-dependent immune responses. Nature. 2005;434:772-777.
7. Ronniolm L, Aim GV. An epitope-specific role for the type I IFN system in SLE. Trends Immunol. 2001;22:427-431.
8. Banchereau J, Pascual V. Type I interferon in systemic lupus erythematosus and other autoimmune diseases. Immunology. 2006;25:383-392.
9. Pascual V, Farkas L, Banchereau J. Systemic lupus erythematosus: all roads lead to type I interferons. Curr Opin Immunol. 2006;18:676-682.
10. Meager A, Visvalingam K, Peterson P, et al. Anti-interferon autoantibodies in autoimmune polyendocrinopathy syndrome type 1. PLoS Med. 2006;3:e289.
11. Zhang L, Barker JM, Babu S, et al. A robust immunospace assay for anti-interferon autoantibodies that is highly specific for patients with autoimmune polyendocrine syndromes type 1. Clin Immunol. 2005;25(suppl):549-555.
12. Pontynen N, Miettinen A, Arstila TP, et al. Aire deficiency causes increased serum levels of interferon-regulated chemokines in the thyroid and increased levels of IFN-α10/IFNα10 in the serum of patients with recent-onset Graves’ disease. Am J Pathol. 2002;161:195-206.
13. Romagnani P, Rotondi M, Lazzeri E, et al. Expression of IFN-α10/CXCL10 and MIG/CXCL9 in the thyroid and in NOD mouse. Eur J Immunol. 2000;30:1884-1893.
14. Perheentupa J. Autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy. J Clin Endocrinol Metab. 2006;91:2843-2850.
15. Zlotz S, Barker JM, Babu S, et al. A robust immunospace assay for anti-interferon autoantibodies that is highly specific for patients with autoimmune polyendocrine syndromes type 1. Clin Immunol. 2006;25(suppl):549-555.
16. Heino M, Peterson P, Sillanpaa N, et al. RNA and protein expression of the murine autoimmune regulator gene (Aire) in normal, Redi−/−deficient and in NOD mouse. Eur J Immunol. 2000;30:1884-1893.
17. Antonelli A, Rotondi M, Fallahi P, et al. High levels of circulating CXC chemokine ligand 10 are associated with chronic autoimmune thyroiditis and hypothyroidism. J Clin Endocrinol Metab. 2004;89:5496-5499.
18. Romagnani P, Rotondi M, Lazzeri E, et al. Expression of IFN-α10/CXCL10 and MIG/CXCL9 in the thyroid and increased levels of IFN-α10/IFNα10 in the serum of patients with recent-onset Graves’ disease. Am J Pathol. 2002;161:195-206.
19. Rotondi M, Falorni A, De Bellis A, et al. Elevated serum interferon-gamma-inducible chemokine-10/CXC chemokine ligand-10 in autoimmune primary myopathy. J Clin Endocrinol Metab. 2005;90:2357-2363.
20. Siivola E, Colgan S, Grossman A, et al. Antibody overexpression of interferon-α10 induces changes in tissue-specific autoantibodies as APECED patients. J Autoimmun. 2006;27:96-104.
21. Meager A, Wadhwa M, Dilger P, et al. Anti-interferon autoantibodies in autoimmune diseases. Trends Immunol. 2005;26:132-138.
22. Romani N, Reider D, Heuer M, et al. Generation of mature dendritic cells from human blood: an improved method with special regard to clinical applicability. J Immunol Methods. 1996;196:137-151.
23. Izrrarry RA, Bolaad BM, Collin F, Cope LM, Hobbs B, Speed TP. Summaries of Affymetrix GeneChip probe level data. Nucleic Acids Res. 2003;31:e15.
24. Smyth GK. Linear models and empirical Bayes methods for assessing differential expression in microarray experiments. Stat Appl Genet Mol Biol. 2004;3:Article3.
25. Wolff AS, Erichsen MM, Meager A, et al. Autoimmunology of the type I IFN system in SLE. Trends Immunol. 2007;28:645-650.
26. de Veer MJ, Holko M, Frevel M, et al. Functional classification of interferon-stimulated genes identified using microarrays. J Leukoc Biol. 2001;69:912-920.
27. Taylor MW, Tsukahara T, Brodsky L, et al. Changes in gene expression during pegylated interferon and ribavirin therapy of chronic hepatitis C virus distinguish responders from nonresponders to antiviral therapy. J Virol. 2007;81:3391-3401.
28. Bonatti E, Baccala R, Theofilopoulos AN. Interferons as pathogenic effectors in autoimmunity. Immunol Rev. 2005;204:9-26.
37. Buttmann M, Merzyn C, Rieckmann P. Interferon-beta induces transient systemic IP-10/CXCL10 chemokine release in patients with multiple sclerosis. J Neuroimmunol. 2004;156:195-203.

38. Krakauer M, Sorensen PS, Khademi M, Olsson T, Setlebjerg F. Dynamic T-lymphocyte chemokine receptor expression induced by interferon-beta therapy in multiple sclerosis. Scand J Immunol. 2006;64:155-163.

39. Migita K, Miyashita T, Maeda Y, et al. Reduced blood BDCA-2(+) (lymphoid) and CD11c(+) (myeloid) dendritic cells in systemic lupus erythematosus. Clin Exp Immunol. 2005;142:84-91.

40. Tomer Y, Blackard JT, Akeno N. Interferon alpha treatment and thyroid dysfunction. Endocrinol Metab Clin North Am. 2007;36:1051-1066.

41. Coccia EM, Severa M, Giacomini E, et al. Viral infection and Toll-like receptor agonists induce a differential expression of type I and lambda interferons in human plasmacytoid and monocyte-derived dendritic cells. Eur J Immunol. 2004;34:796-805.

42. Walker J, Tough DF. Modification of TLR-induced activation of human dendritic cells by type I IFN: synergistic interaction with TLR4 but not TLR3 agonists. Eur J Immunol. 2006;36:1827-1836.

43. Bendriss-Vermare N, Barthelemy C, Durand I, et al. Human thymus contains IFN-alpha-producing CD11c(-), myeloid CD11c(+), and mature interdigitating dendritic cells. J Clin Invest. 2001;107:835-844.

44. Schmidlin H, Donlje W, Groot F, et al. Stimulated plasmacytoid dendritic cells impair human T-cell development. Blood. 2006;108:3792-3800.

45. Vidalain PO, Laine D, Zaffran Y, et al. Interferons mediate terminal differentiation of human cortical thymic epithelial cells. J Virol. 2002;76:6415-6424.

46. Gray D, Abramson J, Benoist C, Mathis D. Proliferative arrest and rapid turnover of thymic epithelial cells expressing Aire. J Exp Med. 2007;204:2521-2528.

47. Strobel P, Murumagi A, Klein R, et al. Deficiency of the autoimmune regulator AIRE in thymomas is insufficient to elicit autoimmune polyendocrinopathy syndrome type 1 (APS-1). J Pathol. 2007;211:563-571.
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