出國報告（出國類別：進修）

困難感染症的新治療策略---如何回復失調的免疫功能以治療罹患後天免疫缺陷成年人之困難感染症

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摘要

易罹患分枝桿菌感染的孟德爾易感性(Mendelian susceptibility to mycobacterial diseases, MSMD)可導致瀰漫性非結核分枝桿菌感染(disseminated nontuberculous mycobacterial diseases)。 2004 年，一研究在成人中描述了由中和抗 IFN-γ 自身抗體(neutralizing anti-IFN-γ autoantibodies)引起的類似免疫缺陷綜合徵。抗干擾素 γ 自身抗體已成為臺灣成人發病免疫缺陷的眾所周知的原因。最近，在一名罹患慢性瀰漫性伯克霍爾德氏菌(*Burkholderia gladioli*)感染的柬埔寨婦女中，找到了一種更為罕見針對白細胞介素 12 (IL12) 的自身抗體(autoantibody against interleukin-12)。當我去 美國 NIH 進修時，我被交代的任務是研究這個病人。我發現該患者的自身抗體不僅中和 IL12，還中和了 IL23。細胞激素 IL12(p35/p40) 和 IL23(p19/p40) 都擁有相同的次級構造(p40)。之後，我定義了這些具有抗 IL12 且也能中和 IL23 的自身抗體，對臨床疾病的重要性和影響程度。再者，在 1250 名罹患有胸腺瘤或嚴重感染的病患中，我篩檢其是否帶有抗 IL12 的自身抗體。然後，我在帶有抗 IL12 的自身抗體組，和未帶有抗 IL12 的自身抗體的多種對照組（包括罹患胸腺瘤患者、感染症患者和健康對照）中，測試其是否帶有抗 IL23 的自身抗體。在 1250 名病患中，有 65 名 (5.3%) 帶有抗 IL12 的自身抗體，其中在 1102 名有接受偵測抗 IL23 的自身抗體的病患中，有 36 名 (3.3%) 帶有抗 IL23 的自身抗體。

在 30 名有血漿檢體保留可用於細胞功能評估，且帶有抗 IL12 的自身抗體的患者中，僅有 26 名 (87%) 可用其抗 IL12 的自身抗體中和 IL12 作用，且 其中 10 名病患雖然帶有抗 IL12 的自身抗體，確沒有發生伺機性感染。在這 30 名病患中，其中 14 名 (47%) 病患帶有抗 IL23 的 自身抗體，可中和 IL23，且這些人都罹患嚴重的分枝桿菌、細菌或真菌的伺機性感染。在剩下 16 位(53%) 未帶有抗 IL23 的 自身抗體的病患中，6 位發生與抗 IL17、IL22 或 IL28 的自身抗體相關的局部感染，而 另外 10 位既沒有發生感染，也沒帶有抗 IL17、IL22 或 IL28 的自身抗體。我的研究結果顯示，我發現可中和 IL23 的抗 IL23 的 自身抗體，其與嚴重且持續感染密切相關，並可在 47% 帶有抗 IL12 自身抗體的成人中檢測到。在不存在抗 IL23 或其下游的作用激素、IL17、IL22 和/或 IL28 的自身抗體的情況下，中和抗 IL12 的自身抗體與病患發生的感染無關。我還發現了兩名只有抗 IL23 的自身抗體，且罹患中樞神經系統感染的病患。因此，我在過去兩年的貢獻是發現

了新的抗 IL23 的自身抗體，這些抗體展現了人類對細胞內和細胞外感染的易感性的一種過往未曾描述過的機制。

除此之外，由於 10% 的新冠(COVID19)重症患者俱有抗干擾素-α 自身抗體，令人驚訝的是，一些患者在不存在這些抗體的情況下，亦可中和抗干擾素-α。 因此，我懷疑他們有針對抗干擾素 α 受體 1 (receptor 1, 這是抗干擾素-α/β/omega 配體[ligands]的常見受體）的抗體。 在兩個罹患嚴重 COVID19 的病患中，藉由使用高通量蛋白質組學測定 (HuProt high-throughput proteomic assay)，證實了我的懷疑。很可惜，我沒有時間在兩年進修結束之前，對這些抗體進行更進一步功能表徵的研究。

目次

一、目的

為對宿主罹患嚴重感染症的易感性有更進一步的了解，並找到更好的診斷工具和治療方法以增強宿主免疫力，而不是只在抗微生物的藥物(即抗生素)使用上一直升級。

二、過程

在美國進修的期間，我致力研究抗細胞激素的自身抗體的特性，和後天免疫缺陷的關係，且確定了至少兩種新的抗細胞激素的自身抗體 ，如下(Tables 1-3 , Fig. 1-4)，幫助建立了國立臺灣大學附設醫院-國立衛生研究院 (NIH) COVID19 合作，如下 (Fig. 5)，建立了印第安納州大學與 NIH 胸腺上皮腫瘤合作 (Indiana University-NIH thymic epithelial tumor collaboration)，並協調和執行了 NIH 工作人員中 COVID19 感染的監測研究 如下(Fig. 6)。

Table 1. Characteristics of individuals screened for anti-IL12p70-binding (anti-IL12) and anti-IL23p60-binding (anti-IL23) autoantibodies between 2007- 2021.

Primary Condition	Total screened for anti-IL12 (*n*)	Anti-IL12 positive *n* (%)	Total screened for anti-IL23 (*n*)	Anti-IL23 positive *n* (%)
Malignant thymoma	66	33 (50.0)	37	20 (54.0)
Opportunistic infections typically linked to IL12-IFNγ deficiency	62	4 (6.5)	16	2 (12.5)
Plasma that inhibited detection of exogenous IL12	5	2 (40.0)	5	2 (40.0)
Autoimmune-polyendocrinopathy-candidiasis-ectodermal-dystrophy	100	3 (3.0)	64	1 (1.6)
Miscellaneous infections or conditions*	835	21 (2.4)	798	9 (1.1)
Healthy controls†	182	2 (1.1)	182	2 (1.1)
Total‡	1250	65 (5.3)	1102	36 (3.3)

Footnotes: The first four groups were screened in a hypothesis-driven manner based on *a priori* knowledge of the protective roles for IL12 in certain intracellular infections and the roles of anti-cytokine autoantibodies in thymoma and autoimmune-polyendocrinopathy-candidiasis-ectodermal-dystrophy (APECED).

*Miscellaneous infections or conditions comprised a group that were screened in an exploratory manner, including those with severe, recurrent (staphylococcal) abscesses, recurrent sinopulmonary infections, deep seated infections, invasive fungal diseases or mucocutaneous candidiasis, other severe viral diseases including life-threatening influenza and acute COVID19. The miscellaneous category included individuals who were subsequently diagnosed with severe combined immunodeficiency due to RAG1/2 deficiency, autoimmune lymphoproliferative syndrome (ALPS) due to *FAS* deficiency, Sjögren syndrome or systemic lupus erythematosus, genetic mutations or variants in the *STAT1*, *IRF8* and *GATA2* genes since patients presenting with immunodeficiency syndromes at the NIH typically undergo anti-cytokine autoantibody profiling and whole exome

sequencing as part of their diagnostic work-up. The very small number of individuals positive for anti-IL12 in this category included: 16 of 754 patients with acute COVID19 (12 mild/moderate disease, 4 critical disease), 1 patient who was subsequently found to have RAG1 deficiency, 3 patients who had no genetic diagnosis, and 1 patient with systemic lupus erythematosus. The number of individuals positive for anti-IL23 in this category included 7 with acute COVID19 (3 mild/moderate disease, 4 critical), 1 of 19 with invasive fungal disease, and 1 with RAG1 deficiency.
†The two healthy controls with low to intermediate anti-IL12 and anti-IL23 were both elderly, Caucasian women (aged 68 and 72 years old) who had donated blood via the NIH Blood Bank, one of whom also tested positive for anti-TNFβ.
‡Not all patients with anti-IL12 had anti-IL23. However, most anti-IL23 containing plasmas had anti-IL12 with 4 minor exceptions; a critical case of COVID19 with borderline negative anti-IL12 and borderline positive anti-IL23 (anti-IL12 FI 9.2 versus anti-IL23 FI 10.3) and three patients (#38, #42) with infections, one with *IL12RB1* deficiency, one with STAT3 deficiency, one undiagnosed with fungal meningoencephalitis, the FIs for anti-IL12 versus anti-IL23 were 6.4 versus 13.0, 7.2 versus 13.6, and 9.7 versus 28.9, respectively. All four actively infected patients also showed IgG binding non-specifically to other cytokines.

Table 2. Clinical characteristics of patients with neutralizing anti-IL12p70 autoantibodies and disease controls without anti-IL12p70 autoantibodies evaluated for neutralizing autoantibodies against IL23.

Patients with anti-IL12 autoantibodies (n = 30)	**ID**	**Age (*y*) M/F**	**Underlying disease**	**Opportunistic infections**	**Daily antimicrobials and outcome**
Group 1A. Anti-IL12+ Systemic (n = 4)	21†	45F	Previously healthy Colitis	Disseminated, persistent *Burkholderia gladioli*, pulmonary aspergillosis	Oral posaconazole, cotrimoxazole, quinolones, TMP-SMX.
	22	14F	Previously healthy	Disseminated, mycobacterial infection, skin and soft tissue "Gram-negative bacterial" infection	Oral itraconazole, TMP/SMX, combination antimycobacterial for years. Currently stable off antibiotics.
	23	40M	Myasthenia gravis	Disseminated *M. genavense* infection	Combination antimycobacterials for years. Currently stable off antibiotics.
	45	41M	Previously healthy	Disseminated persistent coccidioidomycosis, three recurrences over 10 years	Oral fluconazole, high dose.
Group 1B. Anti-IL12+ Localized (n = 13)	05	48M	Thymoma, B2/B3 Myasthenia gravis STAT1 GOF (T224S)	Recurrent *Klebsiella pneumoniae*, pseudomonal sinopulmonary infection, pulmonary NTM, CMC	Parenteral echinocandin, combination antimycobacterials.
	11	41M	Thymoma, B3	Recurrent *Klebsiella pneumoniae/K. oxytoca, Pseudomonas* pneumonia, *Nocardia* peritonitis, VZV, *A. baumannii* bacteremia	Parenteral broad-spectrum antibiotics, died of sepsis after prolonged ICU stay.
	24	41F	Thymoma, B2	Persistent, diffuse tinea corporis, CMC, *Streptococcus mitis* bacteremia without confirmed infective endocarditis, HCAP	Oral fluconazole, terbinafine, and TMP/SMX. Topical terbinafine cream, nystatin gargle, ketoconazole shampoo.
	01	39M	Thymoma, B2	Episodic CAP (hospitalized 2006, 2014), CMC, bilateral VZV (2004, 2008).	Amphotericin gargle.
	13	41F	Thymoma, B2	CAP (hospitalized 2019), CMC, annual VZV (ophthalmic)	Oral fluconazole, valacyclovir.
	08	70F	Thymoma, B3	Fungal pneumonia (*Basidiomycete* most closely resembling *Physisporinus vitreus*)	Died before antifungal treatment.

	25	39M	Thymoma, B2 Myasthenia gravis Granulomatous IBD Vitiligo, alopecia areata	Persistent CMC, esophageal cancer three months before death, recurrent severe VZV, mild COVID19, HCAP	Oral posaconazole, terbinafine, and valacyclovir, supplemented with amphotericin gargle, died of pneumonia after prolonged hospital stay.
	09	45M	Thymoma, B3	Pulmonary cryptococcosis, CMC	Oral fluconazole, nystatin gargle, died of sepsis.
	02	60F	Thymoma, B2 Myasthenia gravis Bronchiectasis (non-smoker)	Persistent fungal sinopulmonary infection (predominantly *Scedosporium apiospermum* positive from 2006-2016), CMC, pulmonary NTM (2006-2007), episodes of pneumococcal and *Pseudomonas* pneumonia	Oral posaconazole (or voriconazole), quinolones, azithromycin TIW, inhaled tobramycin, amphotericin nasal washes, died of exacerbation of underlying diseases.
	26	71M	Thymoma, C Bronchiectasis (non-smoker)	Pulmonary aspergillosis, *Nocardia*, NTM, *Pseudomonas*, CMC	Oral fluconazole (later posaconazole), azithromycin TIW, died of pneumonia.
	27	49F	Thymoma, U Myasthenia gravis Emphysema (smoker)	Pulmonary aspergillosis, *Nocardia*, NTM, *Pseudomonas*, suspected viral myocarditis secondary to CMV	Oral voriconazole, azithromycin, ethambutol, valganciclovir, TMP/SMX, died of unknown cause.
	51	38F	Thymoma, B1 Myasthenia gravis Thrombocytopenia	*Pneumocystis jirovecii* pneumonia (hospitalized)	TMP-SMX.
	52	44F	Thymoma, B3 ITP, IBS	*Pneumocystis jirovecii*, viral myocarditis (ICU)	Oral atovaquone.
Group 1C. Anti-IL12+ CMC (n = 3)	28	79M	Thymoma, U Myasthenia gravis Vitiligo, alopecia areata	CMC, VZV once, severe tinea pedis	Oral fluconazole (or ketoconazole, clotrimazole), oral terbinafine, topical ketoconazole.
	29	80M	Thymoma, U	CMC, CMV/HSV retinitis	Oral fluconazole, valacyclovir, topical ganciclovir (eye drops).
	30†	57M	Thymoma, B1 Myasthenia gravis	CMC	Oral fluconazole.
Group 2. Anti-IL12+ No infections (n = 10)	03	23F	Thymoma, B2 Myasthenia gravis Diabetes mellitus Urticarial vasculitis		Died of progressive malignancy.
	31	53F	Thymoma B2 Myasthenia gravis	VZV once	
	32	66M	Thymoma B3	VZV once	Died of progressive malignancy.
	33	44M	Thymoma B2	Influenza A (pandemic H1N1)	
	34	76M	Thymoma, U		Died of progressive malignancy.
	04	52F	Thymoma, U Myasthenia gravis		Died of progressive malignancy.
	35	41M	Plasmacytoma	Extensive verruca vulgaris (HPV), VZV once	
	18	71M	Thymoma, U	CMV gastritis and viremia	Valganciclovir or foscarnet.

	46	66M	Thymoma, B2 Polycystic kidney disease		Died of progressive malignancy.
	47	35M	Thymoma, B3		Died of progressive malignancy.
Patients without anti-IL12 autoantibodies (n = 21)					
Group 3. Anti-IL12– No infections (n=9)	10	35F	Thymoma, U Myasthenia gravis Langerhans cell granulomatous tumor of frontal skull		
	16	51F	Thymoma, B3		
	06	51F	Thymoma, A		Died of progressive malignancy.
	19	56F	Thymoma, U Good syndrome Pulmonary fibrosis		
	15	39M	Thymoma, C		Died of progressive malignancy.
	14	27M	Thymoma, C Cushing's syndrome s/p bilateral adrenalectomy		
	20	59F	Thymoma, B1 Bronchioalveolar carcinoma Good syndrome Urticarial vasculitis		
	48	70M	Thymoma, B2 Renal cell carcinoma		
	49	68F	Thymoma, B1 Hyperthyroidism		Died of progressive malignancy.
Group 4. Anti-IL12– Infections (n = 12)	37	21M		Disseminated coccidioidomycosis	Parenteral echinocandin, amphotericin B, oral posaconazole, died of sepsis after prolonged hospitalization.
	38	41F	*IL12RB1* deficiency c.1495C>T p.Q499X	Disseminated *Burkholderia cepacia*, intracerebral aspergillosis, gastrointestinal *Mucorales*, HSV	Parenteral echinocandin, vorionazole, liposomal amphotericin B, antibacterials, died with massive gastrointestinal hemorrhage.
	39	41M	Previously healthy Foxp3 variant	Disseminated mycobacterial, CMC, HSV esophagitis *Molluscum contagiosum*	Combination antimycobacterials, acyclovir.
	40	40F	ESRD Sjögren syndrome	Peritoneal tuberculosis, CMC	Parenteral capreomycin, oral moxifloxacin, ethambutol, pyrazinamide for 21 months, fluconazole, clotrimazole gargle, terbinafine cream.
	41	47F	Previously healthy	Recurrent pneumonia, CMC, VZV	Oral posaconazole, amphotericin gargle.
	42	48F	Hyper IgE syndrome STAT3 LOF (1861T>G; p.F621V)	CMC	Nystatin gargle.
	43	34F	Hyper IgE syndrome STAT3 LOF (1144C>T; p.R382W)	CMC, pulmonary aspergillosis, MRSA abscesses	Oral posaconazole or isavuconazole, azithromycin, levofloxacin,

				TMP/SMX, terbinafine or ketoconazole cream, amphotericin gargle.
44	46F	Hyper IgE syndrome STAT3 LOF (1832G>A; p.S611N)	CMC	Oral itraconazole, amphotericin gargle, amoxicillin-clavulanic acid.
36	30M	Previously healthy	Disseminated MAC	Combination antimycobacterials.
32	44F	Hypoparathyroidism	Recurrent sinopulmonary infections (fungal/bacterial sinusitis requiring 3 surgeries), tinea corporis, *Clostridium difficile*	Voriconazole, amphotericin B nasal spray.
50	39M	Psoriatic arthritis	Disseminated coccidioidomycosis, recurrent sinopulmonary infections (3x sinus surgeries), pulmonary aspergillosis	Oral fluconazole for > 2 years. Daily azithromycin+/- levofloxacin.
53	70F		Disseminated coccidioidomycosis	Oral fluconazole.

Footnotes and abbreviations: ID = identification by patient number; M/F = male/female; AAB = autoantibodies; TMP-SMZ = trimethoprim-sulfamethoxazole; Thymoma A, B1, B2, B3, C, U – A, B, C, WHO subtypes "U" for" unclassified"; NTM = non-tuberculous mycobacteria; CMC = chronic mucocutaneous candidiasis including onychomycosis; STAT1 GOF = signal transducer and activator of transcription 1 gain of function mutation; VZV; = varicella-zoster virus reactivation (shingles); HCAP = health-care associated pneumonia; ICU = intensive care unit; CAP = community acquired pneumonia, CMV = cytomegalovirus; HSV = herpes simplex virus; HPV = human papillomavirus; TIW = thrice weekly instead of daily; IL12RB2/1 = interleukin-12 receptor beta-subunit 2/1; ESRD = end=stage renal disease; STAT3 LOF = signal transducer and activator of transcription 3 loss of function missense mutation; MRSA = methicillin-resistant *Staphylococcus aureus*; ITP = idiopathic thrombocytopenic purpura; IBD = inflammatory bowel disease; IBS = irritable bowel syndrome; MAC = *Mycobacterium avium* complex. †Patients 21, 30 have been reported previously in case reports (Refs 17, 24), patients 01 – 17 have been previously reported in our 2010 cohort study, in this study we kept their numbering the same (Ref 13).

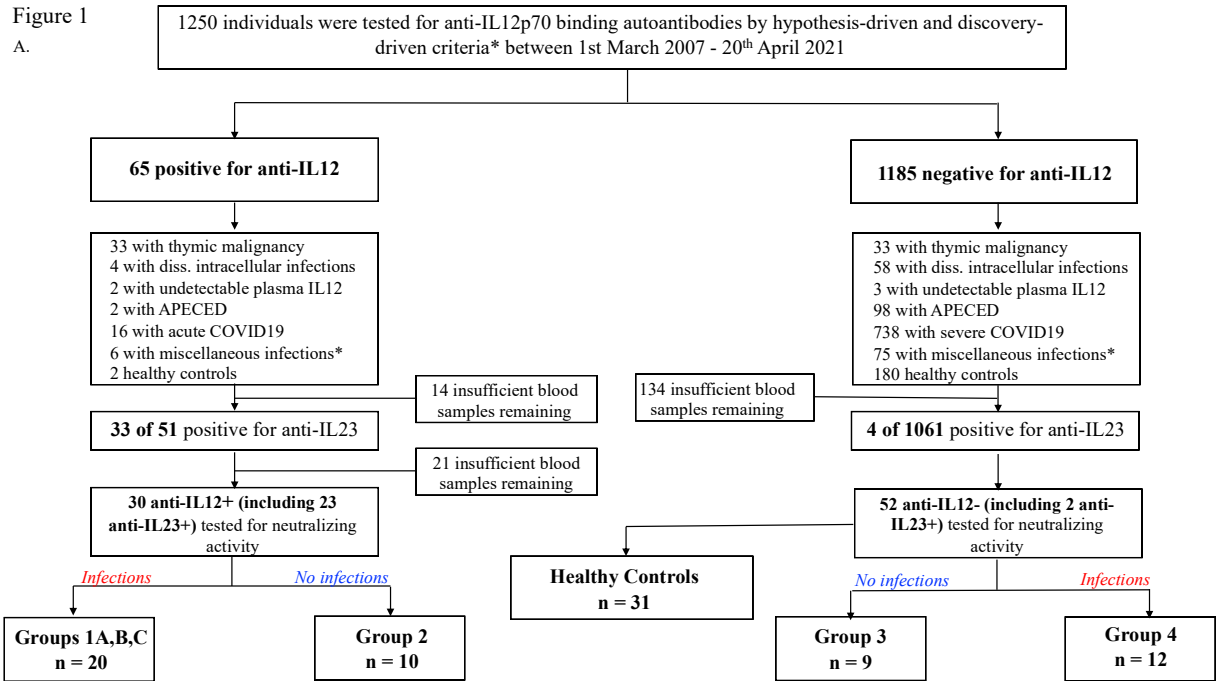
Table 3. Anti-cytokine autoantibodies profile of patient groups with neutralizing anti-IL12p70 autoantibodies opportunistic infections (OIs) with or without and disease controls

Group	Pt (n)	Opportunistic infections (n)	AABs/Pt	IL12 bAAB (%)	IL12 nAAB (%)	IL23 bAAB (%)	IL23 nAAB (%)	IL17A bAAB (%)	IL22 bAAB (%)	IL28 bAAB (%)	IFNα bAAB (%)
1A. Anti-IL12+ Systemic OI	4	Median 2/person Diss. GNB (2) Diss. Mycobac. (2) Pulm. Mold (1)	4	100.0	75.0	75.0	**100.0**	0.0	25.0	0.0	25.0
1B. Anti-IL12+ Localized OI	13	Median 4/person Pulm. GNB (5) Pulm. Mycobac (4) Pulm. Mold (9) Pulm. Nocardia (2) Perit. Nocardia (1) CMC (7) Viruses (7)	7	100.0	84.6	84.6	**76.9**	23.1	53.8	61.5	92.3
1C. Anti-IL12+ CMC only	3	Median 1/person CMC (3) Viruses (2)	6	100.0	66.7	0.0	**0.0**	**66.7**	**66.7**	33.3	100.0
2. Anti-IL12+ No infections	10	Median 0/person Viruses (5)	5	100.0	100.0	90.0	**0.0**	0.0	0.0	20.0	50.0
3. Anti-IL12– Thymoma controls	9	Median 0/person	0	0.0	0.0	0.0	**0.0**	0.0	0.0	11.2	13.3
4. Anti-IL12– Infection controls	12	Median 3/person GNB (1) Mycobac. (3) Mold (6) CMC (5) Viruses (3)	1	0.0	0.0	16.7	**0.0**	0.0	8.3	25.0	10.0

Footnotes and abbreviations: Pt = patient, n= number, AABs = autoantibodies, bAAB (%) = binding autoantibody, percentage positive for IgG that binds the cytokine with a fluorescence intensity that is >10 SD above mean of healthy controls for all cytokines. nAAB (%) = neutralizing antibodies, percentage positive with an $EC_{50}R$ >4. The $EC_{50}R$ is the ratio of EC50 in the presence of patient plasma divided by the EC50 in the presence of healthy control plasma. The effective concentration 50% (EC50) was defined by the concentration at which half of the maximal phospho-STAT response was observed by serial dilutions of the cytokines over the range 0.01-100ng/mL. Diss. = disseminated, GNB = gram-negative bacteria, Mycobac. = mycobacterial, Pulm. = pulmonary, Perit. = peritoneal, CMC = chronic mucocutaneous candidiasis

Figure 1. Outline of Study, Patient Groups and Screening Assays for Anticytokine Autoantibodies

1A. Flow diagram of sequential screening of plasmas for IL12-binding IgG, IL23-binding IgG, before analyzing for anti-IL12 and anti-IL23 biological activity in relation to infectious complications.



*Miscellaneous infections included those with severe or recurrent (staphylococcal) abscesses, fungal infections, recurrent sinopulmonary infections, deep seated infections, parasitic infections, other severe viral infections including life-threatening influenza and, since May 2020, acute COVID19 cases. Beginning in November 2019, we screened individuals prospectively for IL23-binding IgG (n = 980). We characterized the bioactivity of anti-IL12 anti-IL23 autoantibodies for all available samples positive for IL12-binding IgG (n = 30). We included a smaller subset of healthy controls (n = 31) without IL12-binding IgG and also included clinical controls comprising thymoma patients without infections (n = 9) and actively infected controls with a similar spectrum of mycobacterial, *Burkholderia*, or fungal disease (n = 12) for the functional assays.

1B. Multiplex autoantibody binding assay to detect human immunoglobulin G (IgG) against 26-cytokine targets in patient (n = 51) and healthy control plasmas (n = 31) of those included for anti-IL12 and anti-IL23 functional assays and analyzed for infectious outcomes.

Figure 1

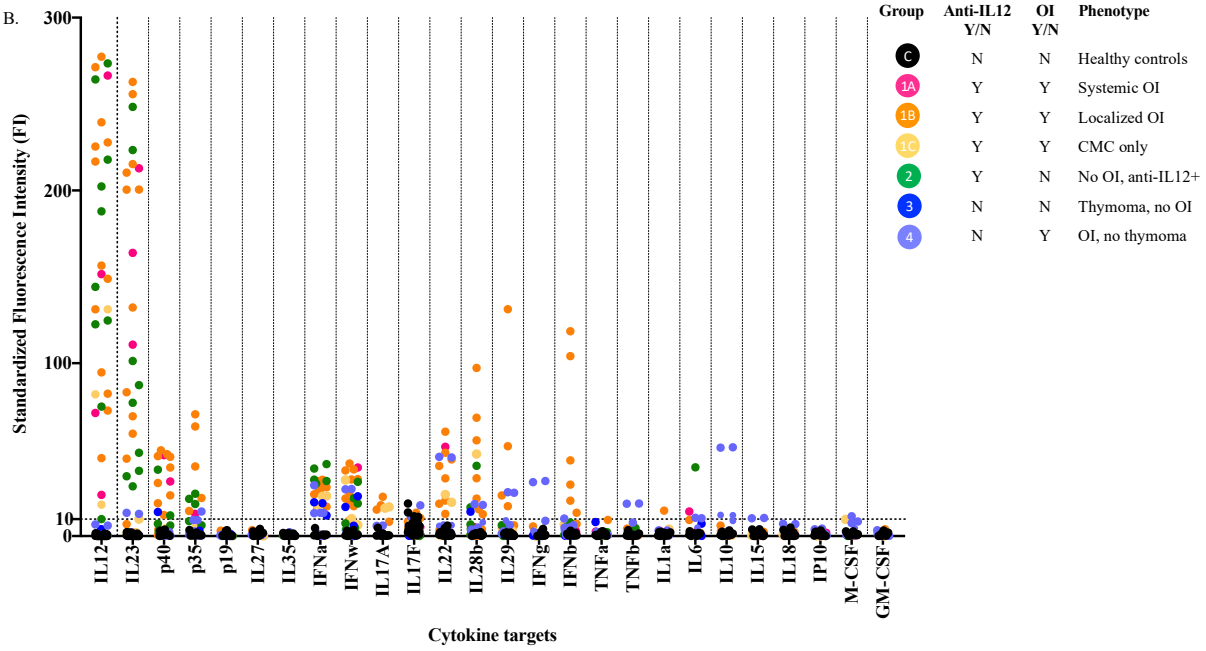
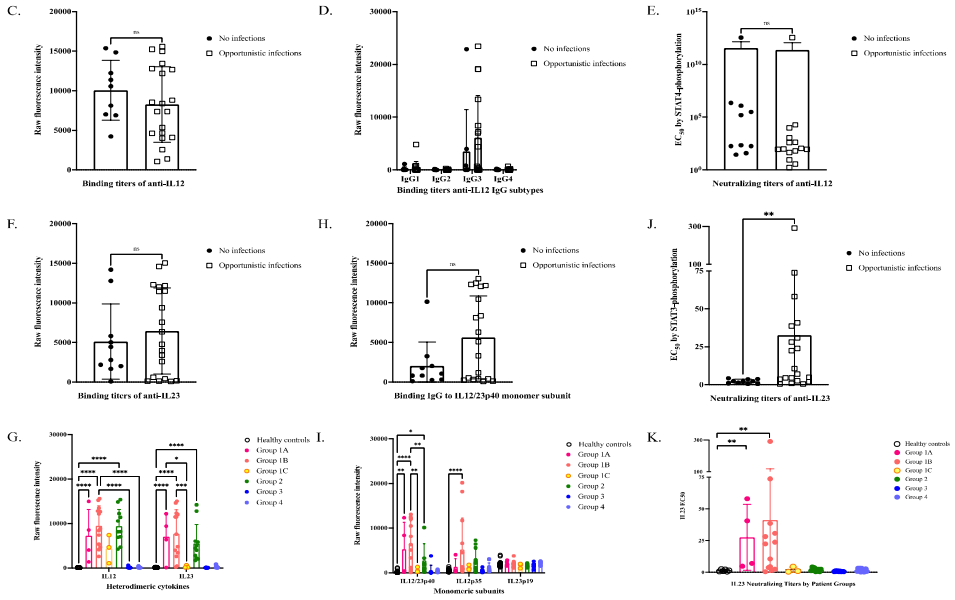


Figure 1



Figures 1C, D, E. Bead-based binding assay showing the anti-IL12 binding IgG fluorescence intensities, the IgG subtypes, and anti-IL12 neutralizing titers between patients with and without opportunistic infections among those with anti-IL12 autoantibodies.

1F. Bead-based binding assay showing the raw anti-IL23 binding IgG fluorescence intensities between patients with and without opportunistic infections among those with anti-IL12 autoantibodies.

1G. Comparing the anti-IL12 and anti-IL23 binding IgG fluorescence intensities among patient groups.

1H. Comparing the binding of IgG to individual subunit monomers of p40, p35, and p19 among patient groups.

1I. Comparing the anti-p40 binding IgG titers between patients with and without opportunistic infections amongst those with anti-IL12 autoantibodies.

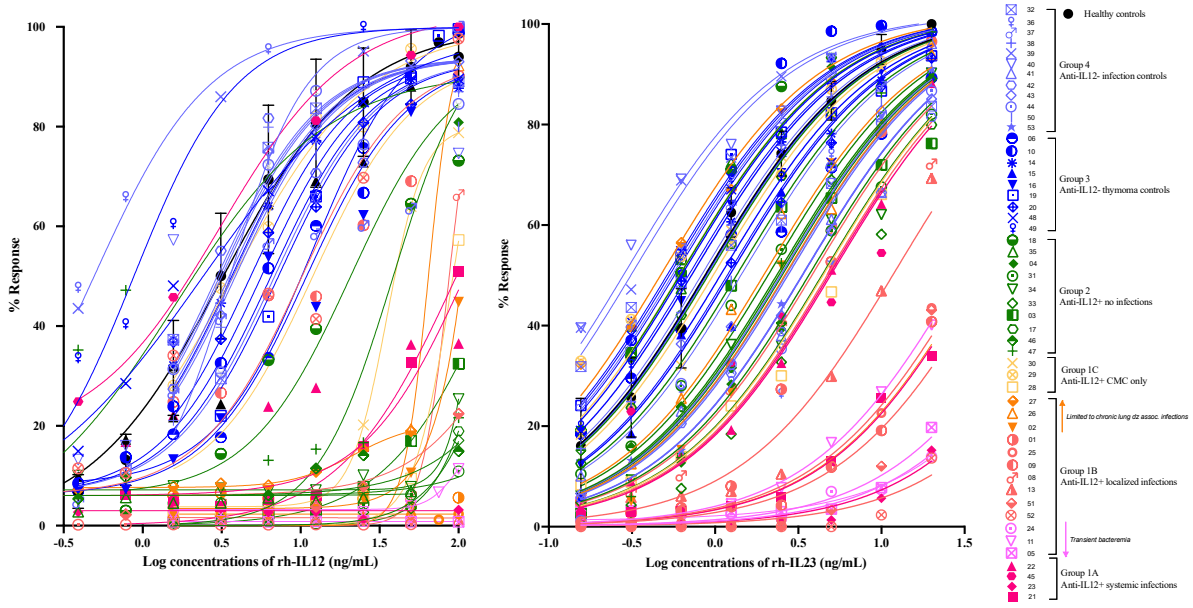
1J. Anti-IL23 neutralizing titers expressed as the effective concentrations of interleukin-23 required to induce 50% of the maximal STAT phosphorylation response are significantly higher in patients with opportunistic infection.

1K. Anti-IL23 neutralizing titers expressed as the effective concentrations of interleukin-23 required to induce 50% of the maximal STAT phosphorylation response correlate with infection severity.

Figure 2. Neutralization Activity Against IL23 and Correlations with Infectious Disease

2A. Individual dose-inhibitory responses against interleukin-12 induced STAT4 phosphorylation (left) and interleukin-23 induced STAT3 phosphorylation (right) by patient groups.

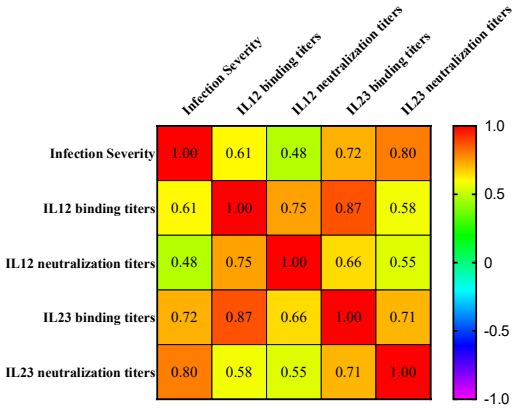
Figure 2 A.



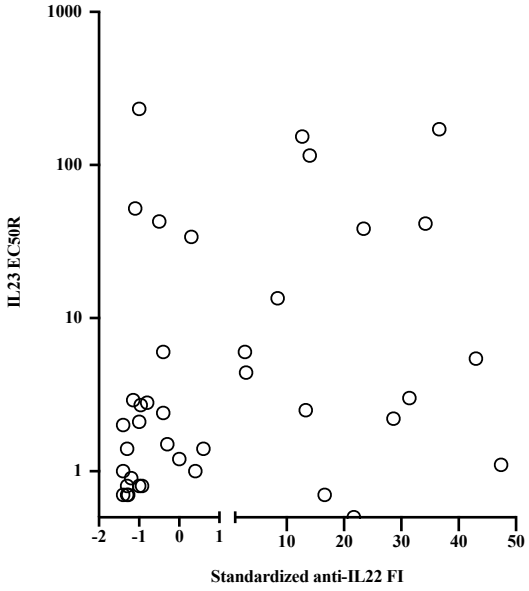
2B. Heat-map showing infection severity correlating with IL23 neutralizing titers (Spearman's rank correlation efficient = 0.80) but much less with IL12 neutralizing titers, or IL12 or IL23 binding titers.

2C. IL23 neutralizing titers against the anti-IL22 binding fluorescence intensities, showing that in the absence of significant IL23 neutralization, patients with infections have anti-IL22 autoantibodies that block downstream of IL23, and the infections tend to be milder (yellow versus pink). Patients without infections neither neutralized IL23 nor bound IL22 (blue, green).

Figure 2 B.

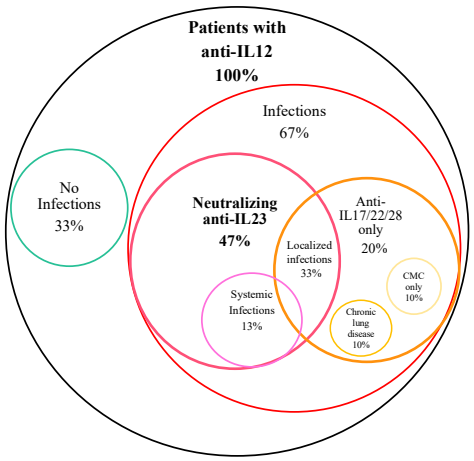


C.



2D. The Venn diagram summarizes the findings that track with infections in this anti-IL12 autoantibody positive cohort.

Figure 2 D.



2E. Neutralizing anti-IL23 autoantibodies inhibits the synergistic induction of IFNγ by IL23 and distinguishes patients with infections from those without severe opportunistic infections (excluding chronic mucocutaneous candidiasis only).

Figure 2 E.

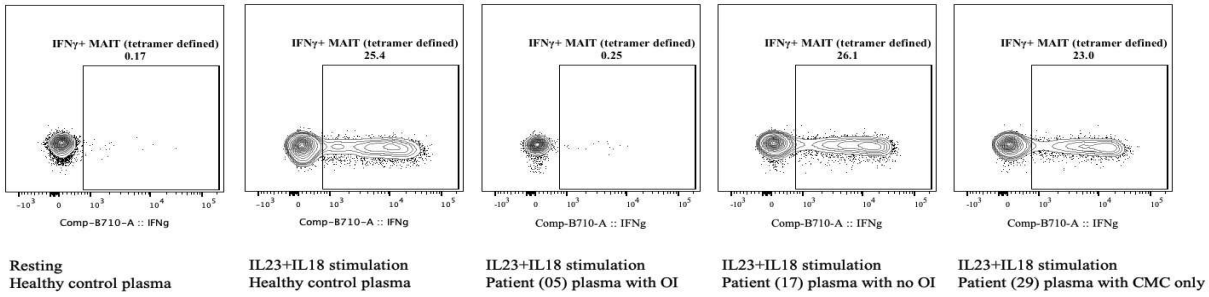
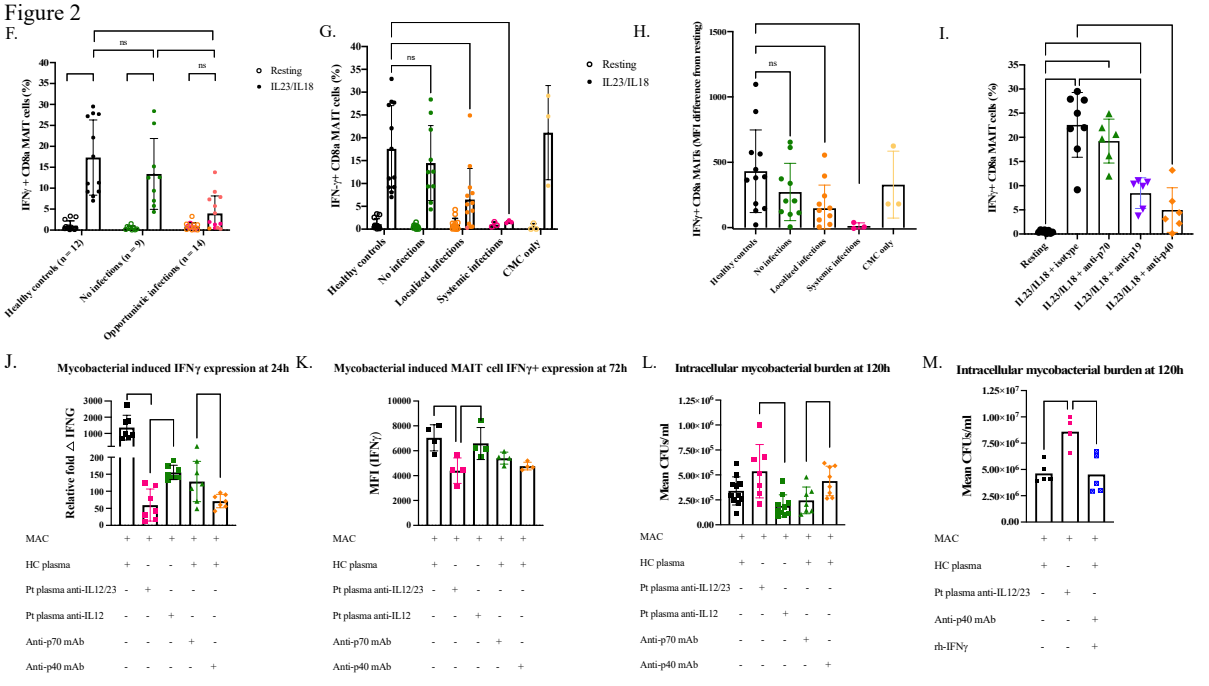


Figure 2



2F. The percentage of IFNγ positive Live+/CD3+/CD8a+/CD161+/MR1-5OP-RU tetramer defined MAIT cells after IL23 and IL18 co-stimulation is increased in the presence of healthy

control plasma or plasma from patients in Group 2 versus Group 1 (excluding chronic mucocutaneous candidiasis only).

2G. Inhibition of IL23-induced IFNγ production by anti-IL23 autoantibodies tracks with severity of opportunistic infections by percentage of IFNγ positive Live+/CD3+/CD8a+/CD161+/MR1-5OP-RU tetramer defined MAIT cells.

2H. Inhibition of IL23-induced IFNγ production by anti-IL23 autoantibodies tracks with severity of opportunistic infections by mean fluorescence intensity of IFNγ in Live+/CD3+/CD8a+/ CD161+/ TCR Vα7.2+ defined MAIT cells.

2I. The IFNγ response in MAIT cells can be blocked with neutralizing monoclonal antibodies against IL23 but not monoclonal antibodies that neutralize only IL12p70.

2J. The IFNγ response of peripheral blood mononuclear cells to live *Mycobacterium avium* can be blocked with neutralizing autoantibodies or monoclonal antibodies against IL12/IL23 more significantly than by monoclonal antibodies that neutralize only IL12p70, as measured by the relative fold change in *IFNG* gene expression normalized to *ACTB* over the uninfected condition at 24 hours post-infection.

The dots represent the average of duplicate wells using healthy control plasmas (n = 7), patient plasmas with anti-IL12 and anti-IL23 autoantibodies (n = 7), patient plasmas with IL12 only autoantibodies (n = 7), healthy control plasmas with 10ug/mL of anti-p40 monoclonal antibody (capable of neutralizing both IL12 and IL23) (n = 7) and healthy control plasmas with 10ul/mL of anti-p70 (IL12-specific) monoclonal antibody (n= 7) performed in two experiments using two healthy donor peripheral blood mononuclear cells.

2K. The IFNγ response of Live+/CD3+/TCRVa7.2+/CD161+ MAIT cells in the presence or absence of neutralizing antibodies against IL12/IL23 to endogenous cytokines induced by *Mycobacterium avium* infecting peripheral blood mononuclear cells.

This plot is generated using healthy control (HC) plasmas (n = 4), patient plasmas with anti-IL12 and anti-IL23 autoantibodies (n = 4), patient plasmas with IL12 only autoantibodies (n = 4), healthy control plasmas with 10ug/mL of anti-p40 monoclonal antibody (capable of neutralizing both IL12 and IL23) (n = 3) and healthy control plasmas with 10ul/mL of anti-p70 (IL12-specific) monoclonal antibody (n= 3).

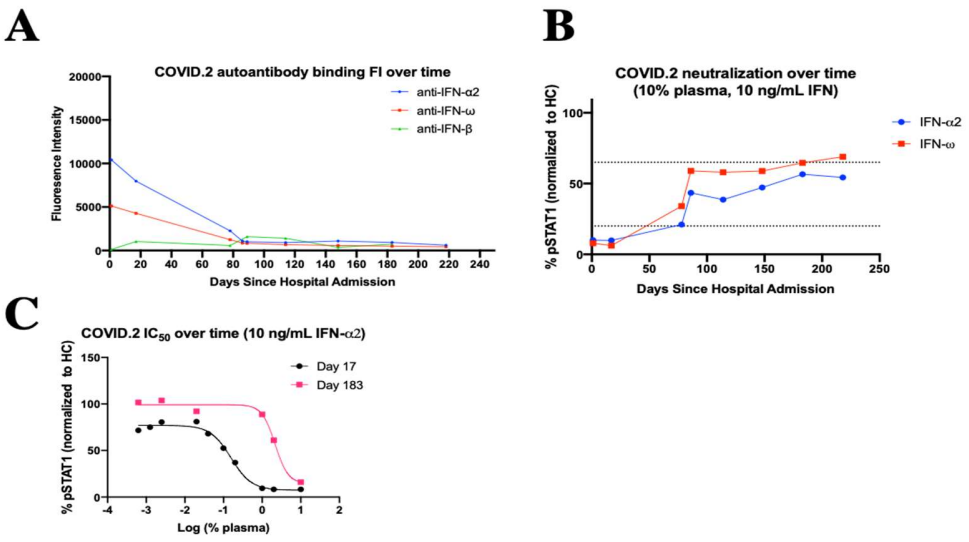
2L. Effect of IL12 single neutralization or dual IL12/23 neutralization on *M. avium* mycobacterial burden in peripheral blood mononuclear cells at day 5 post infection.

The plot is generated from three separate experiments performed in triplicate using 7 healthy control (HC) plasmas, 7 patient plasmas with anti-IL12 and anti-IL23 autoantibodies, and 7 patient plasmas with IL12 only autoantibodies using two different healthy donor peripheral blood mononuclear cells.

2M. Effect of recombinant human interferon-gamma1b (rh-IFNγ) in the presence of an anti-p40 monoclonal antibody on *M. avium* mycobacterial burden in peripheral blood mononuclear cells at day 5 post infection.

The plot is generated from one experiment performed in triplicate using 5 healthy control plasmas, 4 patient plasmas with anti-IL12 and anti-IL23 autoantibodies, and one healthy donor peripheral blood mononuclear cells.

Figure 3. An example of an acute COVID19 patient whose plasma retained the ability to neutralize interferon-alpha even after their anti-interferon-alpha titers have dropped to below the level of detection. A. Anti-IFN-a2, anti-IFN-b, anti-IFN-w autoAb binding titers over time. B. Neutralizing activity of anti-IFN autoAbs against 10 ng/mL of IFN-a2 or IFN-w at 10% plasma over time. C-D. As the patient neutralized 10 ng/mL IFN-a2 and/or IFN-w at 10% plasma at all timepoints, IC_{50} was determined against 10 ng/mL of IFN-a2 or IFN-w at different timepoints to quantify changes in neutralizing activity.



This patient had anti-IFNAR1 binding IgG on HuProt™ assay (see below). IFNa and IFN-b all signal via IFNAR1. So, despite the lack of anti-IFN-b autoantibodies, the patient's plasma still inhibited pSTAT1 signalling induced by IFN-b.

Figure 4. Using a high-throughput proteomic assay (HuProt™) to identify monoclonal antibodies that cluster patients with inherited *AIRE* genetic diseases leading to the syndrome of APECED (autoimmune polyendocrinopathy candidiasis ectodermal dystrophay) in comparison to those with acquired *AIRE* deficiencies such as in thymic malignancies. Patients with known neutralizing autoantibodies to interferon-gamma and GM-CSF were used as positive controls.

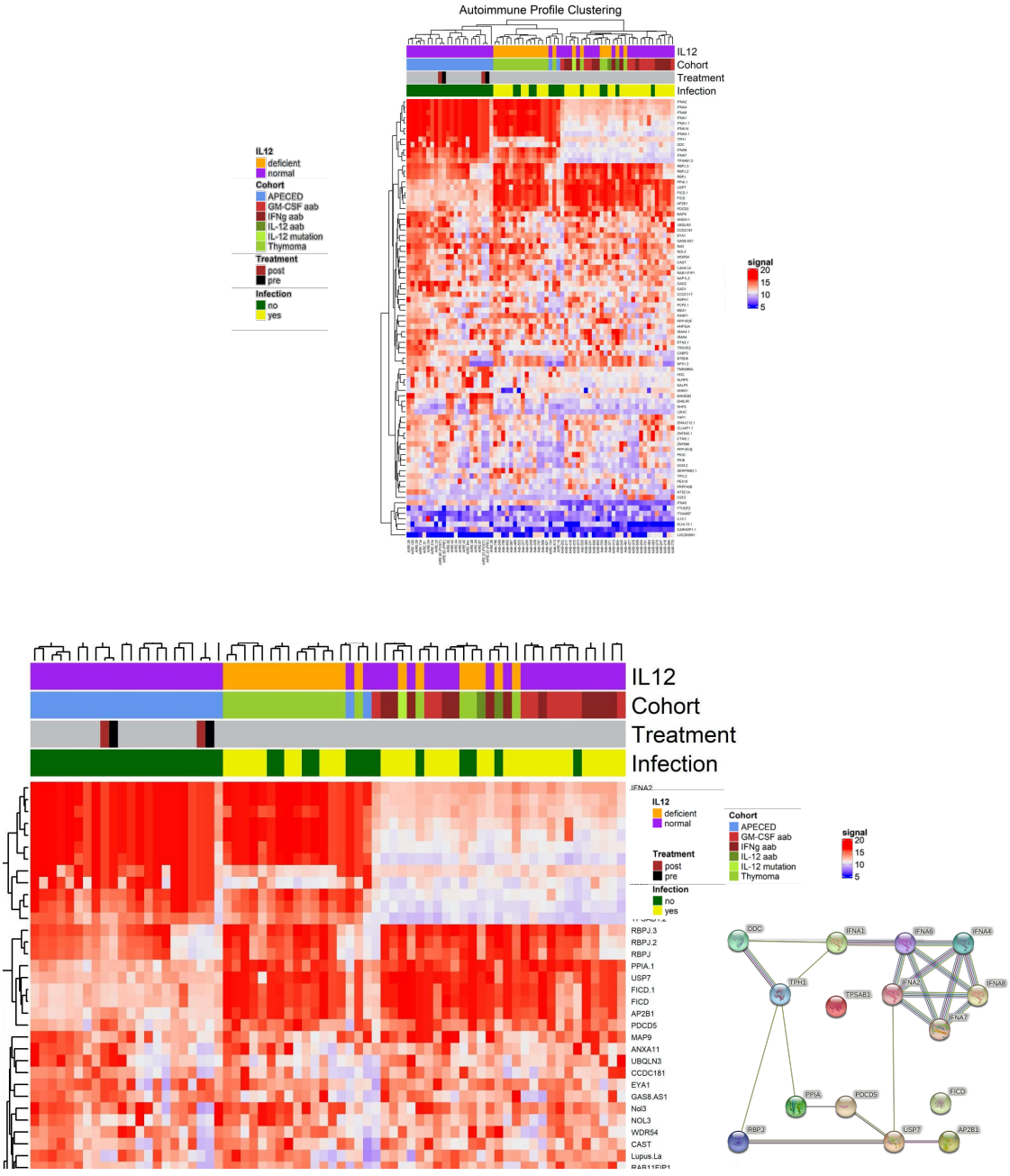
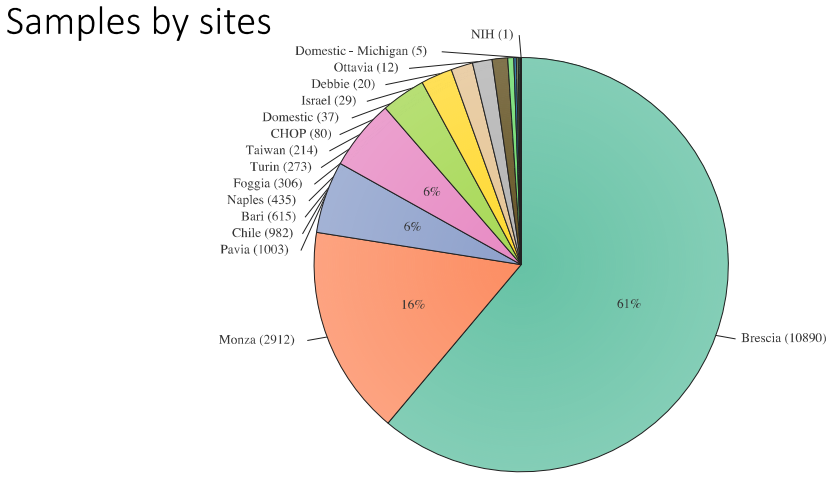


Figure 5. NTUH-NIH COVID19 Consortium (the research is ongoing for this collaboration and the findings remain confidential)

Professor Chang Shan-Chwen, Professor Wang Jann-Tay, Dr. Chen Chien-Yu (Taoyuan General Hospital), Dr. Yang Chia-Jui (Far Eastern Memorial Hospita), Dr. Susan Lee (Veteran's General Hospital, Kaohsiung) agreed to join the consortium after my email invite. Our Taiwanese team helped collect patient samples and sent them to the NIH-NIAID-COVID19 Consortium.



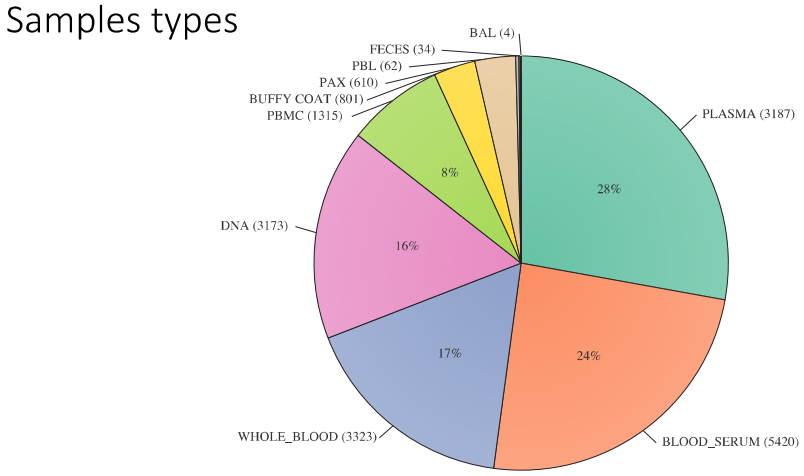


Figure 6a. COVCONTACT Study for NIH Employees _ Outline

Prospective study of asymptomatic COVID19

Primary:

- Correlate results of SARS-CoV-2 RT-PCR using various sample types, antibody assays and culture.

Secondary:

- Determine rate of culture positivity in individuals who persistently shed virus.
- Correlate symptoms with culture positivity and RT-PCR cycle threshold.

Prospective study of asymptomatic COVID19

Subjects:

- Individuals who have recently tested positive or are highly suspected to be positive for severe acute respiratory syndrome-coronavirus-2 (SARS-CoV- 2) infection

Study tools:

- Questionnaire (symptoms/exposure)
- Viral rt-PCR from three sites: NP, mid-turbinate, saliva
- Viral culture: assess whether persistent rt-PCR detection = infectivity
- Serology: Elecsys (Roche) for anti-nucleocapsid protein

Figure 6b. COVCONTACT Study for NIH Employees_Recruitment Summary

Recruitment summary

- N = 18
- Episodes = 19 (one subject enrolled twice)
- First subject visit: 7/22/2020
- Last subject visit completed: 3/22/2021
- All NIH employees (were not successful in enrolling non-NIH employees, FDA employees had expressed interests but restricted access on site)
- All asymptomatic or minimally symptomatic
- 3 had PCR-confirmed SARS-CoV2 on enrollment, 15 had perceived household or workplace exposure

PCR-confirmed cases (n = 3)

- Questionnaires: 2/3 reported persistent ageusia and anosmia (no other symptoms)
- All 3 had persistent positive rt-PCR results only from nasopharyngeal swabs after enrollment (>2 weeks of initial positive PCR test)
- Longest documented interval for positive rt-PCR results: +57 days
- CT values ranged 33.8-38.9, whilst the mid-turbinate and saliva tests rt-PCR tests were negative.
- All simultaneously performed viral cultures were negative for SARS-CoV2.
- All three had positive anti-SARS-CoV-2 nucleocapsid protein

Figure 6c. COVCONTACT Study for NIH Employees_Results Summary

Interesting PCR-positive subject 16

- First enrolled in August 2020. Completed three visits, two weeks apart, no positive rt-PCR, culture or serological evidence of SARS-CoV2 infection.
- Re-enrolled in January 2021, based on positive rt-PCR results at NIH.
- Was persistently positive by rt-PCR only for his NP samples from 1/6/21- 2/11/21.
- Vaccinated on 1/27/21 in between his 2nd and 3rd visits.
- He had qualitatively positive antibodies at his 1st visit on 1/6/21
- We have banked plasma to do quantitative serology at some point.

No prior PCR-confirmed tests (n= 15)

- Questionnaires: common- flu like symptoms including fever, rhinorrhea, sore throat, myalgia, fatigue
- One reported a positive lateral flow test
- None tested positive by rt-PCR (all 3 sites sampled in all subjects)
- Herpesvirus-like virus was recovered in the Vero culture of one saliva specimen of a SARS-CoV2 PCR negative subject
- No SARS-CoV-2 virus was cultured
- None had positive anti-nucleocapsid protein

2020 年 3 月和 2020 年 6 月，當我們的實驗室處於封鎖狀態時，我自願在汽車測試線上對 COVID19 進行鼻咽測試。我學會瞭如何進行細胞培養、處理人類血液樣本、對血漿樣本進行多重自身抗體測定、進行細胞因子刺激和中和測定、進行共聚焦顯微鏡檢查、定量 PCR、流式細胞術、ELISA、體外感染測定、訪問他們的臨床、遺傳，並研究數據庫並分析使用不同軟件程序（如 PRISM、FlowJo、Imaris 和 Morpheus）生成的數據。我想使用這些基本技能來診斷我們患者的未知免疫缺陷。

三、心得

此次進修前，我並沒有科學和實驗室背景。所以在美國進修的這段期間，我必須非常努力地學習和工作，才能習得可靠的實驗技術的能力，並贏得我進修時期老闆和同事對我的科學領域發現的信任。美國因新冠疫情導致學校和工作長期封鎖，對我的家人和我來說，都是精神上的一大挑戰。我希望我有更多的時間來完成我在科學領域上的發現。儘管如此，我很高興至少我已改變 NIH 荷蘭實驗室的做法。他們現在已常規篩檢抗 IL23 自身抗體，並採用我設計的 mucosal-associated-invariant-T-cells (MAIT) 細胞功能分析方法，作為他們的標準分析方法，如上圖 (Fig. 2E-G)。

四、建議事項

起初希望兩年進修可以延長，因在美國 COVID19 大流行的期間，我進修的實驗室被封鎖了幾個月，只能在家遠距離工作。然而，臺灣在 2021 年 5 -6 月爆發的 COVID19 似乎表明是時候回歸了。希望感染科團隊可以向風免疫和血腫同事們學習如何使用免疫調節劑來治療免疫致病性自身抗體。感謝臺大醫院和感染科部門的支持與幫助。