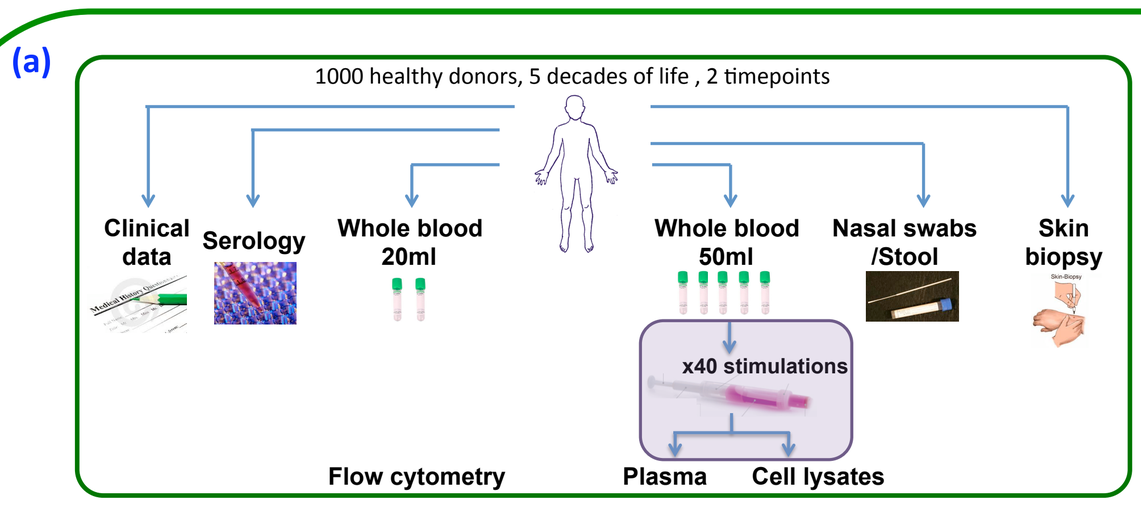
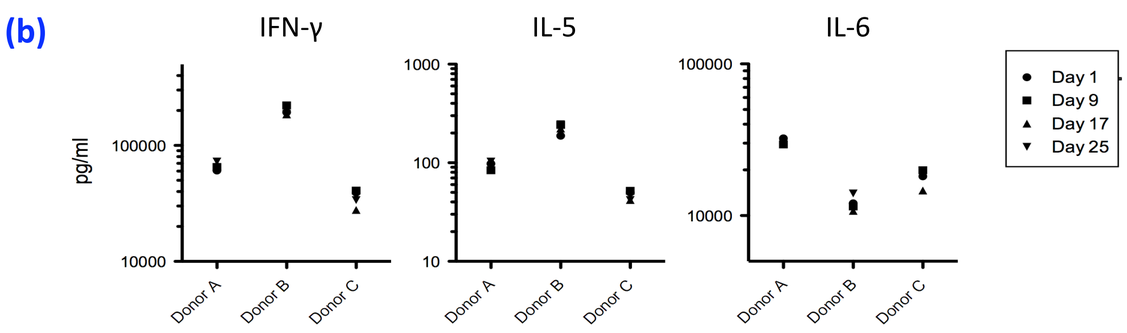
Characterization of the healthy immune response to innate sensor specific stimuli

Darragh Duffy, Vincent Rouilly, Valentina Libri, Milena Hasan, Benoit Beitz, Mikael David, Alejandra Urrutia, Sam La Brie, Annick Dubois, Cécile Delval, Stéphanie Thomas, Lars Rogge, Manfred Schmolz, Lluis Quintana-Murci, Matthew Albert and The *Milieu Intérieur* Consortium

Centre d'Immunologie Humaine

Institut Pasteur





(c)

Stimulus	Abbreviation	Concentration	Supplier	Sensor / Receptor
Null	Ø		NA	
HK *E.coli 0111:B4*	HKEC	10^7 bacteria	Invivogen	complex
HK *S. aureus*	HKSA	10^7 bacteria	Invivogen	complex
HK *L. rhamnosus*	HKLR	10^7 bacteria	Invivogen	complex
BCG (Immucyst)	BCG	$3 * 10^5$ bacteria	Sanofi Pasteur	complex
HK *H. pylori*	HKHP	10^7 bacteria	Invivogen	complex
HK *C. Albicans*	HKCA	10^7 bacteria	Invivogen	complex
Influenza A Virus (live)	IAV	100 HAU†	Charles Rivers	Complex
Sendai virus (live)	SeV	10 HAU	Charles Rivers	Rig-I & Mda/5
FSL-1	FSL	2 μg/ml	Invivogen	TLR2/6
Poly I:C	pIC	20 μg/ml	Invivogen	TLR3
LPS-EB (ultrapure)	LPS	10 ng/ml	Invivogen	TLR4
Flagellin-ST	FLA	0.25 μg/ml	Invivogen	TLR5
Gardiquimod	GARD	3 μM	Invivogen	TLR7
R848	R848	1 μM	Invivogen	TLR7 & TLR8
ODN 2216	ODN	25 μg/ml	Invivogen	TLR9

Figure 1. 1000 healthy donor population based study to characterise a healthy immune response
(a) The outline of the *Milieu Intérieur* study design and samples that were collected.
(b) Reproducibility data showing the cytokine (IFN-γ, IL-5, IL-6) response of 3 donors following True Culture™ LPS stimulation at 4 different timepoints over 25 days.
(c) The list of microbe and TLR specific stimuli selected for whole blood stimulation (additional stimuli were included in the study, not shown here)

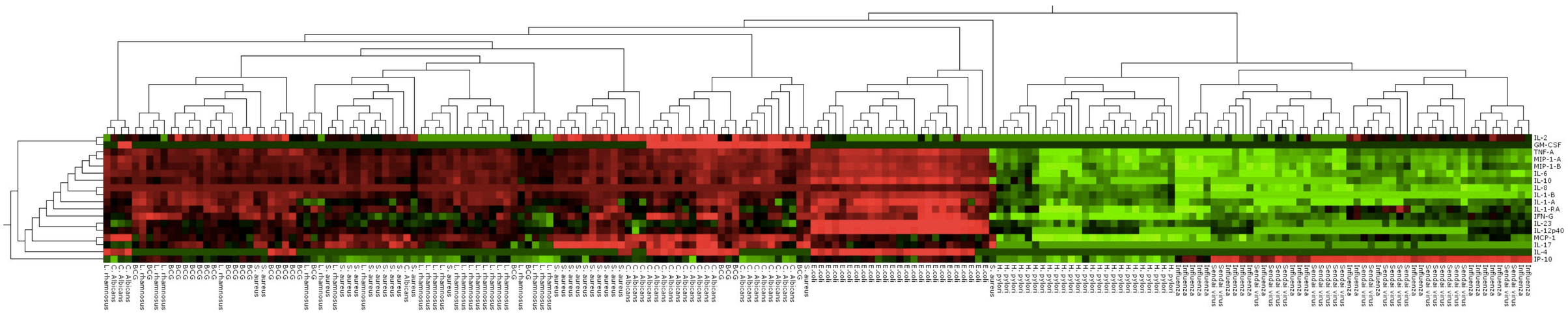


Figure 2. Unique inflammatory signatures induced by complex microbial stimulation
Hierarchical clustering of the 18 most differential proteins selected by ANOVA (q-value $<10^{-30}$) following whole blood stimulation of twenty-five donors (twelve men, thirteen women; ages 30-39) with heat-killed *Escherichia coli*, *Staphylococus aureus*, *Lactobacillus rhamnosus*, *bacillus Calmette-Guérin* (BCG), *Helicobacter pylori*, *Candida Albicans*, Influenza A virus (H1N1), and Sendai virus.

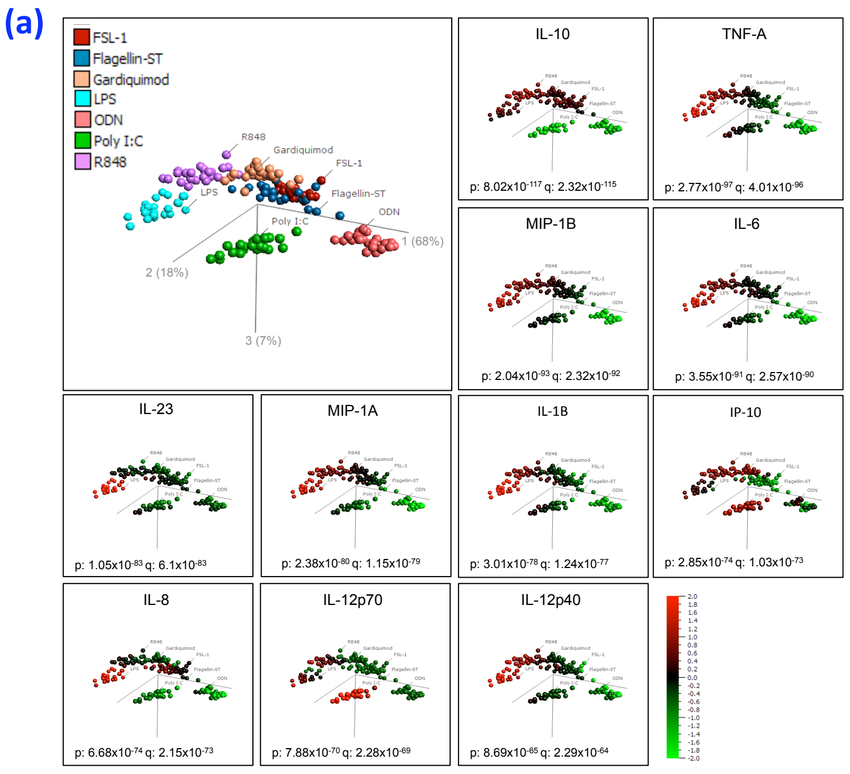
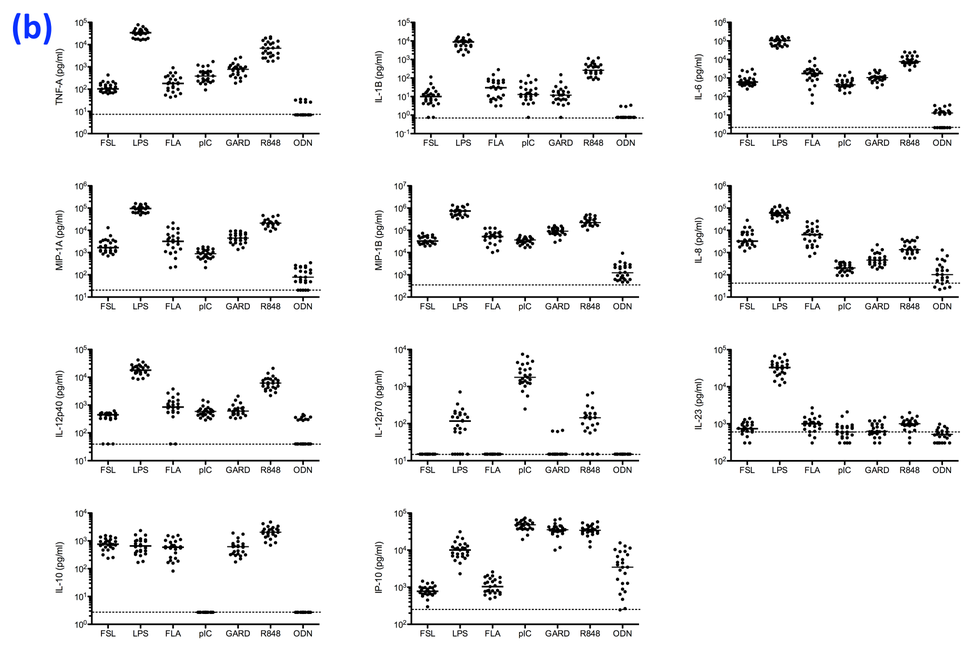


Figure 3. Segregation of TLR agonists based on their induced protein signatures.
(a) Principal component analysis (PCA) of the protein response following whole blood stimulation with TLR agonists (FSL, pIC, LPS, FLA, GARD, R848, ODN). Each colored circle represents a different whole blood stimulation condition; and the PCA was run using the eleven most differentially induced proteins (cutoff value was determined by ANOVA, q-value $< 10^{-60}$). The PCA plot shown captures 85% of the total variance within the selected dataset. Expression levels for each of the eleven protein analytes was overlaid on the PCA plot. A heat map indicates the relative expression of the indicated protein analyte (red indicating high expression levels, green low expression levels). p- and q-values are reported. **(b)** Dot plots of the protein response to whole blood TLR stimuli for the 11 most differential analytes that were identified.



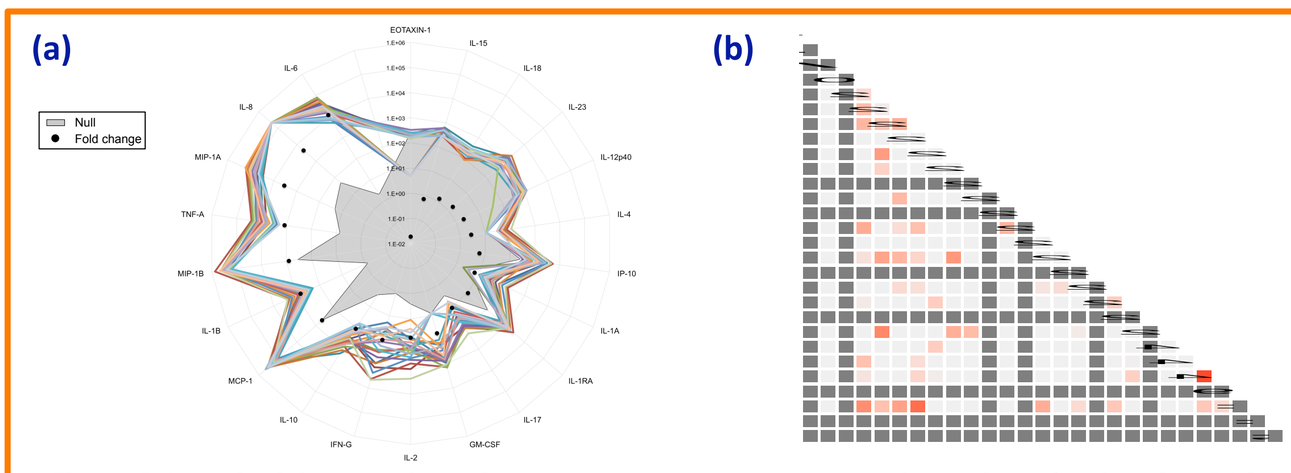
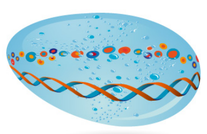


Figure 4. Inter-individual variance in the whole blood response to stimulation with *Candida Albicans*.
(a) Radar plot of the induced response to *Candida Albicans*. Analytes represented as pg/ml and ordered clockwise in increasing fold change (compared to null). Each donor is represented by a colored line, connecting the concentration of measured protein analytes. The grey polygon depicts the median value of the null response & black dots indicate the fold change as compared to the null response. Analytes with a median fold change (stimulation/null) > 1.3 or < -1.3 were included. **(b)** correlation matrix was constructed to define the relationship between the individual proteins.

Summary

1. **True-Culture™ (Myriad RBM) whole blood stimulation systems allows the definition of induced inflammatory signatures for a broad range of innate and adaptive stimuli.**
2. **The induced protein response to 8 medically relevant bacteria, fungi and viruses was identified.**
3. **The unique immune response to 7 different TLR agonists could be identified based on the response of just 11 proteins when applied to principle component analysis.**
4. **Up to 3 log fold differences were observed between donors for certain stimuli induced responses.**



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