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Minireview

Transcriptional profiling of vaccine-induced immune responses in humans and non-human primates

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Summary

There is an urgent need for pre-clinical and clinical biomarkers predictive of vaccine immunogenicity, efficacy and safety to reduce the risks and costs associated with vaccine development. Results emerging from immunoprofiling studies in non-human primates and humans demonstrate clearly that (i) type and duration of immune memory are largely determined by the magnitude and complexity of the innate immune signals and (ii) genetic signatures highly predictive of B-cell and T-cell responses can be identified for specific vaccines. For vaccines with similar composition, e.g. live attenuated viral vaccines, these signatures share common patterns. Signatures predictive of vaccine efficacy have been identified in a few experimental challenge studies. This review aims to give an overview of the current literature on immunoprofiling studies in humans and also presents some of our own data on profiling of licensed and experimental vaccines in non-human primates.

Introduction

Vaccination remains the most cost-effective measure to prevent infectious diseases, and many are readily preventable through induction of protective immunity especially functionally active antibodies, e.g. diseases caused by bacterial toxins, several viruses and some encapsulated bacteria (Plotkin, 2010). For many other important pathogens, such as *P. falciparum*, *M. tuberculosis*, *Human Immunodeficiency Virus* and *S. aureus,* however, correlates of protection are either not known, or it is unclear how to best induce potent polyfunctional immune responses that are broad and long-lived. These are major obstacles to rational vaccine design that have resulted in numerous failed clinical trials and are major contributors to the increasing costs of vaccine development.

In preclinical studies, the total and functional antibody titres as well as T-cell responses (IFN gamma secreting CD4 and CD8 cells) induced by the vaccine are usually measured prior to taking a candidate vaccine into clinical trials. However, results obtained in rodents and occasionally even in non-human primates are often not predictive of the vaccine's efficacy in humans. Even if a vaccine proves effective, side-effects due to local and systemic inflammatory reactions may lead to termination of its development. Unfortunately, there is currently only a limited set of biomarkers available to predict safety in humans based on animal experimentation (e.g. pyrogenicity). There is thus a pressing need for the identification of biomarkers to guide the preclinical and clinical development of vaccines. To this end, 'systems biology' – an emerging discipline that employs bioinformatics to computationally model molecular networks – is increasingly being applied to study the complex immunological responses to vaccination in order to define genetic signatures of immune cells, which are predictive of efficacy and safety of vaccines (Oberg *et al.*, 2011). The term 'systems vaccinology' has been coined for this approach (Pulendran *et al.*, 2010). This mini-review provides an overview of recent results of the immunoprofiling of various vaccines in humans *in vivo*, aiming to identify common gene signatures, which could be applied to vaccine development. In addition, preliminary results from our own profiling studies in non-human primates of a number of licensed vaccines are presented.

Profiling of human immune responses to vaccination

In the past few years gene expression profiling has been utilized to capture a global view of the post-vaccine immune response. PBMC or whole blood of humans immunized with a number of different vaccines have been

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examined including live attenuated yellow fever vaccine (YF17D) (Gaucher *et al*., 2008; Querec *et al*., 2009), live attenuated measles vaccine (Haralambieva *et al*., 2010), live attenuated *Francisella tularensis* vaccine (Fuller *et al*., 2007), live attenuated tuberculosis vaccine (BCG) (Fletcher *et al*., 2009), live attenuated and trivalent subunit influenza vaccines (Zhu *et al*., 2010; Bucasas *et al*., 2011), and a recombinant subunit malaria vaccine (RTS,S) (Vahey *et al*., 2010). The latter study is particularly interesting as vaccinees were subjected to challenge with live parasites, permitting investigation of gene signatures associated with vaccine efficacy. Similar pre- and post-challenge expression profiling has been done in non-human primates, as well. In one such study, rhesus macaques were immunized with adenovirus-vectored HIV vaccines and challenged with simian immunodeficiency virus (Palermo *et al*., 2011). Salient features of these studies will be discussed below.

Yellow fever

In the yellow fever vaccine (YF17D) studies, early post-vaccination gene signatures predictive of CD8+ T cell responses and neutralizing antibody responses were identified with 90% and 100% accuracy respectively (Querec *et al*., 2009) and transcription networks regulated by STAT1, IRF7 and ETS2 were shown to be involved in multi-functional and persistent immune responses (Gaucher *et al*., 2008). Specifically, the gene encoding the growth factor BAFF/BLyS (a TNF-like cytokine that supports survival and differentiation of B cells) was present in all 15 gene signatures collected during two independent Yellow fever 17D immunization trials and correlated with the induction of neutralizing antibody responses. The key genes in predictive signatures for CD8+ T cell responses were SLC2A6, which encodes GLUT1, a membrane protein regulating glucose transport and glycolysis, and EIF2AK4, which is associated with stress response and phosphorylation of the translation initiation factor eIF2α. Other genes involved in the stress response were found to be upregulated as well, prompting the hypothesis that the induction of an integrated stress response in the innate immune system might play a key role in shaping the CD8+ T cell response to yellow fever vaccine (Pulendran *et al*., 2010).

Measles

In a study comparing cytokine secretion patterns and gene expression, PBMCs isolated from donors who had been immunized against measles were stimulated with different measles virus vaccine or wild-type strains. It was found that the highly attenuated measles virus strain Edmonston tag induced Th1 and Th2 T cell responses as well as innate and inflammatory cytokine responses (Haralambieva *et al*., 2010). In contrast, viruses expressing a functional wild-type phosphoprotein (P) gene elicited predominantly inflammatory cytokine responses, characterized by moderate to low levels of IL-6 and IL-1β secretion, while suppressing other cytokine responses including IFNs. These data support previous findings that wild-type paramyxovirus P, V, and C proteins inhibit IFNα/β induction and IFN (α/β or γ) signalling by interfering with different transcription network regulators such as STAT and JAK1, or by interacting with MDA5 to block dsRNA binding.

Tularaemia

A study profiling immune responses to an intradermally inoculated, live attenuated *F. tularensis* vaccine (LVS) in adults revealed that the most pronounced changes in PBMC gene expression occurred at early post-vaccination time points ($\leq$ 48 h), and most immune related genes that were upregulated followed this pattern (Fuller *et al*., 2007). Robust early upregulation of pro-inflammatory and innate-immunity related genes, such as IL-18, IL13-RA1, IRAK-3, CD39, CD116 and several TLRs (notably 4 and 8), is indicative of functional responses such as phagocytosis, exocytosis, super oxide formation, antigen processing, cytokine/chemokine production, and signal transduction. In aggregate, these data indicate a strong activation of dendritic cells and other innate immune system cells. However, the largest number of immune related genes was found to be downregulated early. This pattern suggests that numerous genes are selectively up- or downregulated in a timeframe consistent with innate effector activation, with resultant modification of the environment in which immune cell activation will take place, and possibly dampening an overshooting inflammatory response. Subsequent upregulation of genes in the late post immunization period (8 and 14 days p.i.) corresponded temporally to acquired immune effector activation; however, of the late upregulated genes, only one was immune-related – LCK (lymphocyte cell-specific protein tyrosine kinase, involved in surface receptor signalling in NK, NKT and T-cells). Most genes upregulated at the late time point have biosynthetic and metabolic functions, which is consistent with upregulation of a coordinated immune response. Nine of 42 genes with a pattern of sustained upregulation throughout the 14 days of the study were directly linked to immune function. Among these were genes associated with innate defence mechanisms such as regulators of endocytosis/phagocytosis, granule exocytosis, chemotaxis and inflammatory cytokines. Intradermal injection of LVS results in formation of a pustule, clearly visible by 18 h p.i., which eventually ulcerates. While the humoral response to LVS, as measured by microagglutination assay, was highly

variable among the five vaccinees, transcriptome analysis revealed that the vaccine elicited a consistent response across all genes and at all time points in all vaccinees (correlation > 0.88).

Tuberculosis

Live attenuated *mycobacterium tuberculosis* vaccine (BCG), which is given intradermally at birth, was immunoprofiled in PBMC isolated from five infants 10 weeks post immunization. Interestingly, purified protein derivative of tuberculin (PPD) and live BCG induced similar gene expression profiles in isolated PBMC characterized by upregulation of genes associated with the classic, pro-inflammatory macrophage response (IL-6, GM-CSF, IL1F9) and downregulation of leucocyte genes. In general, a larger number of genes was found to be downregulated rather than upregulated. Notable among the downregulated genes were genes of the peroxisome proliferator-activated receptor (PPAR) signalling pathway, which is involved in activation of the alternative, anti-inflammatory macrophage response. Thus, a particular combination of suppressed and upregulated genes may be key in determining immunity to TB (Fletcher *et al*., 2009).

Influenza

Several studies have profiled the immune responses to trivalent inactivated influenza vaccine (TIV) and to live attenuated trivalent vaccine (LAIV). Two distinct temporal patterns of gene expression in response to immunization with TIV were observed in 92 adults (Bucasas *et al*., 2011). The early phase of transcriptional activation (24 h post immunization) was characterized by upregulation of genes whose products act on host viral sensing through TLR7 and TLR8, resulting in significantly increased transcript levels of genes participating in the antiviral defence response, cellular activation and differentiation (e.g. IFN response pathway, JAK/STAT signal transducers, NF-κB). In contrast, the late response (2 weeks p.i.) was characterized by upregulation of genes involved in cellular proliferation. The abundance levels of 494 transcripts correlated significantly with the haemagglutination titre response index. Interestingly, the difference between expression of the genes STAT1 and E2F2 alone generated a gradient that corresponded clearly to the titre response index at the two extremes of the response spectrum. Thus, early upregulation of interferon and antigen presentation pathways are associated with a higher antibody response. In contrast, the majority of upregulated genes and enriched functional pathways in low responders were not specifically related to cell-mediated immune responses.

In another study the immunogenicity and regulation of recall responses was evaluated by a series of clinical studies performed during the annual influenza seasons in three consecutive years, in which a total of 56 young adults were immunized either with TIV or LAIV (Nakaya *et al*., 2011). Profiling was done in PBMC and FACS sorted subsets of immune cells. In addition, cell type-specific transcriptional signatures were discerned in the PBMC microarray analyses, based on a meta-analysis of publicly available microarray studies in which the gene-expression profiles of isolated individual cell types of PBMCs (T-, B-cells, monocytes, NK cells, etc.) had been analysed. Antibody responses to vaccination (HAI titres) were much higher in TIV than LAIV recipients. In subjects vaccinated with TIV, myeloid DCs and B cells had the most differentially expressed genes (DEGs), whereas in subjects vaccinated with LAIV plasmacytoid DCs had the most DEGs. The molecular signature induced by vaccination with LAIV was characterized by genes encoding molecules closely associated with the interferon signalling pathways, such as STAT1, STAT2, TLR7, IRF3 and IRF, with the greatest difference in gene expression observed at day 3 post immunization. Cell-type specific analysis showed high expression of most interferon-related genes in monocytes and natural killer cells. In contrast, the signature induced by vaccination with TIV was characterized by upregulation of genes encoding two transcription factors, XBP-1 and ATF6B, which are central to regulating the unfolded protein response. This was detectable in sorted antibody secreting cells, consistent with the requirement of these cells to enhance their capacity to secrete large amounts of correctly folded immunoglobulins. Interestingly, genes with high expression included TNFRSF17 (encoding BCMA, a member of the BAFF-BLyS family of receptors), whose expression has been shown before to be a key feature of the best predictive signatures of neutralizing antibody responses to YF-17D (Querec *et al*., 2009). In total, 42 sets of gene signatures (3–4 genes each) were identified by discriminant analysis via mixed integer programming (DAMIP) to predict 'high' or 'low' responders to TIV vaccination (> fourfold HAI titre increase) with an estimate of correct classification > 85%. To test the hypothesis that this approach can identify novel mechanisms of immune regulation, the authors investigated the role of the gene CaMK4, whose expression was negatively correlated with HAI titres on day 28. CaMKIV is known to be involved in T cell development, inflammatory responses and the maintenance of haematopoietic stem cells, but its role in B-cell responses is not known. *In vitro* stimulation of mouse and human splenocytes with TIV resulted in phosphorylation of CaMKIV, suggesting that the vaccine may trigger activation of CaMKIV, and immunization of wild-type and $CaMK^{-/-}$ mice resulted in threefold to 6.5-fold higher antibody titres in the knock-out mice (Nakaya *et al*., 2011). These results suggest that CaMKIV is important in the regulation of the B cell response.

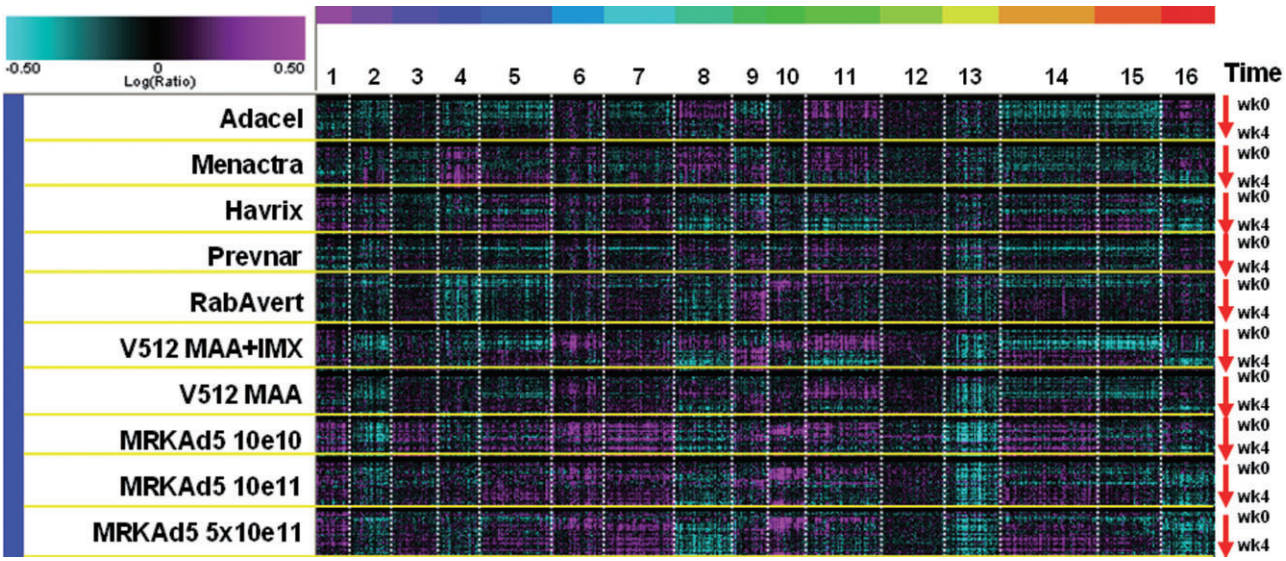

Fig. 1. Overall blood gene signatures in non-human primate immunized with seven marketed or experimental vaccines. Genome-wise gene expression data were collected from 8 time points including 0 h (baseline), 4 h, 8 h, 24 h, 1 week, 2 weeks, 3 weeks and 4 weeks post vaccination. For each vaccine formulation, a one-way ANOVA was performed to select for signature genes modulated at any one of 7 time points post vaccination in comparison to the baseline at $P \leq 0.001$. The heatmap shows expression values in Log(Ratio) of 23 653 sequences derived from the union of 10 ANOVA gene lists. The signatures are robust and both common and unique signature clusters can be identified among tested vaccines. For examples, genes in clusters 6, 10 and 11 are generally upregulated in all tested vaccines at day 1 (i.e. 4, 8 and 24 h post vaccination) although the intensities are variable among vaccines; genes in clusters 9 and 14 are upregulated at later time points (weeks 2–4) whereas genes in clusters 2 and 13 are generally downregulated. There are also vaccine-specific gene signatures such as cluster 4, which is specifically upregulated by Menactra and cluster 1, which is predominantly upregulated by MRKAd5 and Havrix.

Malaria

To date, two studies have reported immunoprofiling that identified gene signatures or signalling pathways, which were correlated with vaccine efficacy against experimental challenge. In one study, volunteers receiving three doses of adjuvanted RTS,S vaccine (*Plasmodium falciparum* circumsporozoite sequences fused to the hepatitis B surface antigen) were challenged by the bites of infected mosquitoes. Genes associated with host inflammatory response, apoptosis, and the protein kinase cascade were upregulated within 24 h of the third vaccination, regardless of the outcome after challenge. However, gene set enrichment analysis performed on samples obtained 2 weeks after the third vaccination revealed that upregulation of genes in the proteasome degradation pathway (PSME2, PSMB9, PSMB6, PSMA4) was associated with protection (Vahey *et al.*, 2010). These genes play complex roles in the efficiency of MHC peptide processing and antigen presentation.

HIV

In an AIDS vaccine trial in rhesus macaques, the animals were primed twice (orally plus intranasally, followed by intratracheally) with a replication competent adenovirus 5 vector, which expressed human immunodeficiency virus (HIV) envelope protein, simian immunodeficiency virus (SIV) gag protein or SIV nef protein, alone or in combination with two intramuscular boosts with recombinant HIV gp140 protein or an HIV peptomer in monophosphoryl lipid A stable solution (Palermo *et al.*, 2011). Animals were challenged intravenously with a $SHIV_{89.6P}$ challenge stock, and peak viral load and time to viral set point were measured. While at peak viraemia the expression profiles of the immunized animals were very similar, the best-protected animals showed upregulation of genes related to B-cell development and lymphocyte survival pre-challenge; increased expression of IL-27 and complement components post-challenge were identified as possible mechanisms of protection. Divergent expression profiles at the viral set point for the different immunized groups implied distinct immunological responses despite phenotypic similarities in viral load and CD4+ T-cell levels.

Head-to-head comparison of vaccine-induced gene signatures in the blood of non-human primates

A whole-blood gene expression database comparing the response of non-human primates to a panel of marketed (Adacel, Menactra, Havrix, Prevnar and RabAvert) and experimental vaccines are shown in Fig. 1. Among the experimental vaccines, V512 is an influenza vaccine based on the ectodomain of the M2 protein conjugated to Outer Membrane Vesicles of *Neisseria meningitidis* serotype B11; MRKAd5-gag is an HIV vaccine based on a

replication deficient adenovirus type 5 expressing HIV gag. Genome-wide gene expression data for each vaccine were generated from 4 rhesus monkeys at 8 separate time points (pre-vaccinated baseline, post-vaccination at 4, 8, 24 h, and days 7, 14, 21 and 28). One-way ANOVA analysis was performed individually for each vaccine and used to generate a heat map for the union of 10 ANOVA-derived signatures (Fig. 1). In addition, the blood module approach (Chaussabel *et al*., 2008) and the metagene model (Huang *et al*., 2003) for identifying gene signatures were employed to obtain gene signatures. Annotation was performed using pathway analysis tools such as Ingenuity (Ganter and Giroux, 2008).

In general, all vaccines induced robust blood gene signatures at the ANOVA *P*-value selected (> 20 000 sequences in Fig. 1) with clear time-dependent kinetics. Each vaccine generated a unique gene expression pattern across time points although some relatively common features can be detected. For example, clusters 6, 10 and 11 (Fig. 1) contain genes associated with innate immunity, cytokine production and responses to virus infection, including IFN-inducible genes. These genes were upregulated by all vaccines at early time points and decreased thereafter; similar gene sets were induced by yellow fever and influenza vaccines as reported previously (Gaucher *et al*., 2008; Querec *et al*., 2009 and Bucasas *et al*., 2011). The extent of upregulation induced by the experimental vaccines (i.e. V512/influenza and MRKAd5-gag) was generally higher than by the marketed vaccines. Clusters 9 and 14 include genes whose expression was downregulated at early time points but was increased after one week. These genes are linked to T cell receptor signalling, cell cycle progression, and response to stress. Gene clusters predominantly modulated by certain vaccines were also observed; for example, clusters 1 and 7, which are induced mainly by the MRKAd5-gag vaccine and to lesser extents by the V512/influenza and Havrix vaccines, point to genes associated with the ubiquitin pathway, protein folding and mitochondrial dysfunction. Clear vaccine dose-dependent expression patterns and adjuvant-induced signatures were found with the three different doses of MRKAd5-gag vaccine and V512 influenza vaccine with or without ISCOMATRIX™ respectively (Fig. 1).

More detailed pathway engagement information was acquired when each vaccine was examined separately. For instance, at early time points, Adacel administration activates key innate immunity-related pathways including Toll-like receptor (TLR), GM-CSF and FcγR signalling and downregulates adaptive immunity-related pathways including T cell receptor, NK cell, and CCR5 signalling. The expression patterns of these innate immunity-related versus adaptive immunity-related signalling pathway genes reverse between early (4–24 h post vaccination) and later time points (1–2 weeks post vaccination), which is consistent with the general vaccine-induced gene expression patterns shown in Fig. 1. This type of analysis may provide a framework for hypothesizing how a particular vaccine works to exert its protective activity.

We also identified pre-defined gene modules as well as *de novo* gene clusters, which are correlated to a combined adverse event scores derived from human clinical data (manuscript in preparation). In another internal preclinical study, we identified another set of gene modules and signatures, which are either positively or negatively correlated to antibody titres from monkeys vaccinated with an experimental vaccine formulated with a panel of test adjuvants. We observed a consistent result with a recently published article of Nakaya and colleagues (2011), who showed that T cell receptor signalling genes are negatively correlated to TIV Ab titres in humans.

Conclusion and outlook

Results emerging from profiling of immune responses to vaccination suggest that the type and duration of immune memory are largely determined by the magnitude and complexity of the innate immune signals that prime the acquired immune responses. As evident from the *in vivo* profiling studies, the extent of upregulation of immune-related and especially interferon regulated genes is generally higher following administration of live attenuated vaccines than inactivated vaccines. However, early upregulation of pro-inflammatory and innate immune genes seems to be off-set by concomitant or subsequent downregulation of several immune related genes, possibly to dampen overshooting inflammatory reactions. Some general principles of successful priming of the innate immune system are evident from vaccination studies performed in humans, and in several cases the same 'genetic master switches' of antigen processing and presentation, as well as B-cell and T-cell activation, have been found (Table 1); however, gene signatures predictive of specific adaptive immune responses vary widely in these studies, as would be expected from the variety of vaccine compositions, adjuvants and routes of inoculation used in these studies, not to mention variation introduced by the different methodologies used to analyse the data. An important question is therefore whether common genetic signatures can be identified that will predict immunogenicity of different vaccines. To this end the recent comparison of inactivated trivalent influenza vaccine (TIV) to live attenuated Yellow fever 17D vaccine (YF-17D) is very interesting. For both vaccines high expression of TNFRSF17, which encodes BCMA, was found to be part of gene signatures predicting the development of high titres of functional antibodies (Querec *et al*., 2009; Nakaya *et al*., 2011).

Table 1. Overview of published vaccine immunoprofiling studies in humans and non-human primates.

Vaccine (composition)	Dosing/route Subject/sample	Gene signature characterization	Pathway/end-point correlation	Additional notes	Reference
Yellow fever 17D (live attenuated virus)	1×, s.c. 40 adults, whole blood	– 594 genes at $P < 0.05$, $\|FC\| > 1.3$ from days 3, 7, 10, 14, 28 and 60 post vaccination – Signature peak at days 3 and 7 with genes regulated mainly by IRF7, STAT1 and ETS2	Upregulated pathways: – TLR, IFN, B cell activation, antigen processing and presentation, inflammasome and complement pathways – DC-, NK- and macrophage-associated genes Downregulated pathway: – ribosomal genes	– Increased IL-1β production – Increased proliferation of B cell, NK cell and macrophage – Variable YF-specific T cell response among subjects – Mixed Th1/Th2 response – Consistent *in vitro* response – Data validated by 2 qPCR studies in 3 independent cohorts	Gaucher *et al.* (2008)
Yellow fever 17D (live attenuated virus)	1×, s.c. 15 adults, PBMC	– 97 genes modulated in > 60% subjects by a factor of + 0.5 (Log_2) on days 3 or 7, followed by ANOVA and fold change cut-off (for days 0, 1, 3, 7 and 21) and corrected with Benjamini and Hochberg $P < 0.05$ – Signature genes regulated by IRF7, SREBF1 and transcription factors bind to ISRE	Upregulated pathways: – IFN, anti-viral, complement, antigen processing, STAT, ubiquitination, cytoskeletal, cell adhesion, chemotaxis and poly (ADP-ribose) polymerase pathways CD8+ T cell response-correlated signature: – 839 genes enriched in metabolism and immune response genes (e.g. EIF2AK4 and SLC2A6)	– Increased IP-10 and IL-1α – Increased percentages of mDC, pDC and monocyte – Variable YF-specific T cell and neutralizing Ab response among subjects – C1qB and EIF2AK4 predict YF-CD8 T response with 90% accuracy – TNFRS17 predicts neutralizing Ab response with 100% accuracy	Querec *et al.* (2009)
Measles virus (MV) Edmonston tag (MVEdmtag) (live attenuated virus) Also tested wild-type MV (MVwt) and MVEdmtag expressing wt P gene (MVwtP)	Hx of routine measles immunization (2×, s.c.) 179 adolescents (12–18 years) PBMC *in vitro*	– 30 cytokine or related genes upregulated > fourfold with $P < 0.05$ by MVEdmtag 24 h after treatment – MVwt and MVwtP induced only 4 and 11 genes respectively – 27 genes downregulated (> fourfold) by MVwt and 22 genes downregulated (> fourfold) by MVwtP when compared with MVEdmtag respectively	Up regulated genes by MVEdmtag: – IFNα, IFNγ, IFNλ1, IL-1β, IL-2, IL-6, IL-8, TGFβ, IL-10, TNFα, EIF2AK4, MX1 and OAS1 – IFN-induced antiviral genes (e.g. CSF1, CSF2, IL1A, IL1F5, IL7, IL15, TGFA, INHBA, EIF2AK2, TNFSF10 and TNFSF13B)	– Not a genome-wide microarray *in vivo* study. Assessed only 88 cytokine genes *in vitro* – MVEdmtag induced higher secretion of IL-2, IFNγ, IL-10, IL-1β, IL-6, TNFα, IFNα and IFNλ1/IL-29 – wt V protein significantly, and P protein to a less extent, attenuates the induction of type I, II and III IFNs in lymphocyte and monocyte cell lines	Haralambieva *et al.* (2010)
Francisella tularensis vaccine (LVS) (live attenuated bacterium)	1×, i.d. 5 adults (22–54 years) PBMC	– ~450 genes at paired t-test $P < 0.01$ and $\|FC\| > 2.0$ for 18 and 48 h post vaccination – High gene expression correlations (> 0.95) among subjects within each group (pre-vaccination, early (18 and 48 h) and late (days 8 and 14) post vaccination)	Up early: Innate immunity, TLR signalling and Ag presentation (e.g. FCGR2A, IL18, IL13RA1, IRAK3, NCF2, DCL-1 and MHC genes) Up late: LCK Sustained up: LRP1, M6PR, TXNIP, VAMP8, MAPKAPK3, SPN, CMRF-35H/Irp60, ARRB1 Down early: T cell signalling, cytokine response and proliferation (e.g. CD96, CCL5, TNF-RSF25, CD3D)	– Variable *F. tularensis*-specific Ab response among subjects – No attempt to correlate gene signature with Ab titre – Small cohort with only 5 subjects and no testing cohort	Fuller *et al.* (2007)

Table 1. *cont.*

Vaccine (composition)	Dosing/route Subject/sample	Gene signature characterization	Pathway/end-point correlation	Additional notes	Reference
Bacillus Calmette-Guerin (BCG) (live attenuated bacterium)	1×, i.d. 5 newborns (10-week-old) PBMC *in vitro* Infants vaccinated with BCG at birth. PBMC obtained 10 weeks after and challenged with BCG or PPD *ex vivo* for 12 h	– 411 genes modulated by BCG at $P < 0.01$, 136 up- and 275 downregulated – 291 genes modulated by PPD at $P < 0.01$, 95 up- and 196 downregulated – BCG and PPD signatures similar to each other (258 genes in common. $r = 0.965$) and not separated by *k*-means clustering – More genes were downregulated than upregulated	Pathways modulated: – Cell adhesion molecules (downregulated by BCG only) – PPAR signalling (downregulated by both) – Cytokine-cytokine receptor interaction – Haematopoietic cell lineage – TLR signalling – JAK-STAT signalling – Graft-versus-host disease Up regulated genes: IL-1β, IL-6, IL-8, TNF-α, IP10, MIP-1β, GM-CSF, IL1F9 and MDC Down regulated genes: CD36, PPAR-γ, RXR, FABP4, M-CSF receptor, GSN and TGF-β1	– Magnitude of response BCG > PPD – Few genes differentially expressed > 2× between BCG and PPD – Development of M1 macrophage in vaccinated infants – M2-associated PPAR-γ pathway is downregulated – TB vaccine efficacy assessed by IFNγ protein. IFNγ mRNA up 2.12× by PPD and 2.19× by BCG	Fletcher *et al.* (2009)
Influenza TIV [purified subunit protein (HA, NA), no adjuvant]	1×, i.m. 92 healthy male adults (18–40 years) whole blood	– 3854 RefSeq and 242 non-annotated transcripts with FDR cut-off of 0.01 on the p value for the day effect (pre- and days 1, 3 and 14 post vaccination) – Top 1% differentially expressed genes include IFN-inducible, STAT signalling and ribosomal genes TRI-correlated signature: – A titre response index (TRI)[a] based on the titre changes for the 3 Abs was constructed for vaccine responsiveness – Expression levels of 494 transcripts correlated with TRI[a]	Early (day 1) upregulated pathways: – Ag processing and response to organism – Immune process – Positive IL-6 regulation – JAK/STAT signalling – Response to IFNγ and NF-κB pathway Late (days 3 and 14) upregulated pathways: – Cell and RNA metabolic process – Ribosomal subunit and translational elongation – Macromolecular biosynthesis; T cell selection Downregulated pathways (all time points): – Release of NF-κB; AP-1 complex – RNP complex; dephosphorylation TRI-correlated pathways: Ag presentation; Activation of IRF by cytosolic pattern recognition receptors; IFN signalling and TREM1 signalling	– This study has a larger sample size with sufficient power to detect significant correlations between transcript levels and Ab response – The development of TRI permits a statistical significant classification of trivalent flu vaccine recipients on the basis of responsiveness – Expression of STAT1, IRF9, SPI1, CD74, HLA-E, TNFSF13B, PRDX2, PRDX3, E2F2, PTEN and ITGB1 can be used to predict TRI but STAT1 and E2F2 expression alone correponds to TRI at the 2 extremes of response spectrum	Bucasas *et al.* (2011)

Table 1. *cont.*

Vaccine (composition)	Dosing/route Subject/sample	Gene signature characterization	Pathway/end-point correlation	Additional notes	Reference
Influenza TIV [purified subunit protein (HA, NA), no adjuvant]	1×, i.m. 28 adults (18–50 years) PBMC	– 2650 transcripts (1594 up- and 1056 downregulated) with |FC| > 1.25 in > 20% subjects, then applied ANOVA $P < 0.05$ (Benjamini Hochberg adjusted), signal-to-noise and significance analysis of microarray (SAM) – Differentially expressed genes mostly in myeloid DCs and B cells	1772 genes correlated with HAI titre Pathways negatively correlated to HAI titre: TCR signalling, CD28 and iCOS/iCOSL signalling in Th cell, IL-3 signalling, CTLA4 signalling in CTL Pathways positively correlated to HAI titre: NK cell signalling, NO and ROS production in macrophage, leucocyte extravasation and TREM1 signalling Gene correlated to low HAI titres: CAMK4 Genes correlated to high HAI titres: Genes regulated by XBP-1 and ATF6B (IGHE, IGHG3, IGHG1, IGHD, TNFRSF17)	– Microarray performed in 4 cell types: CD19+ B cells, CD14+ monocytes, myeloid DCs and plasmacytoid DCs – Signature validated in 2 additional cohorts with 90% accuracy – Camk4 KO mice induced enhanced Ag-specific Ab titres with TIV vaccination – TNFRSF17 and CD38 predict both TIV and YF vaccine Ab titres	Nakaya *et al.* (2011)
Influenza LAIV [live attenuated virus (cold adapted)]	1×, i.n. 28 adults (18–50 years) PBMC	– 5309 transcripts (3114 up- and 2195 downregulated) with |FC| > 1.25 in > 20% subjects, then applied ANOVA $P < 0.05$ (Benjamini Hochberg adjusted), signal-to-noise and significance analysis of microarray (SAM) – 828 up- and 617-downregulated genes in common with TIV – Differentially expressed genes mostly in plasmacytoid DCs	Common LAIV- and TIV-induced pathways: Inflammatory and antimicrobial responses which contain TLR5 and TLR8 and inflammasome genes Type 1 IFN-related genes induced by LAIV: STAT1, STAT2, TLR7, IRF3, IRF7, OAS1, IRF7, MX2	– Significantly different signatures observed between TIV and LAIV – Most subjects vaccinated with TIV were high HAI responders whereas most subjects vaccinated with LAIV were low HAI responders – Many IFN-related genes modulated by LAIV but not by TIV	Nakaya *et al.*, (2011)
Influenza LAIV and TIV [live attenuated (cold adapted) and trivalent inactivated virus]	1×, i.n. 85 children (12–35 months), 43 with LAIV and 42 with TIV Whole blood samples	– 265 genes with |FC| > 2 in more than 25% of LAIV- or TIV-vaccinated subjects 7 days post vaccination – 6 coexpressed gene cluster (A–F) identified	– Cluster A: IFNγ-stimulated genes – Cluster B: erythrocyte development genes downregulated by GM-CSF – Cluster C: CD4 T cell differentiation genes and genes driven by E2F -Cluster DEF: IFN-inducible/ -related genes (IRF7, IFIT1, –2, –3, OAS1, –2, 3, MX1, MX2, IFI44)	– No analysis of correlation to Ab titre – Results consistent with Nakaya and colleagues (2011), i.e. type I IFN-inducible genes were activated more significantly by LAIV than by TIV 7 days post vaccination	Zhu *et al.* (2010)

Table 1. *cont.*

Vaccine (composition)	Dosing/route Subject/sample	Gene signature characterization	Pathway/end-point correlation	Additional notes	Reference
Malaria RTS,S [recombinant fusion protein (CSP peptides – HbsAg) adjuvant AS01 or AS02]	3×, i.m. 39 adults PBMC obtained at baseline, day of 3rd vaccination, 24 h, 72 h, 2 weeks after vaccination and day 5 after challenge	– 63 genes with Significance Analysis of Microarrays FDR < 1%. These genes were upregulated at 24 h and resolved at 72 h after 3rd vaccination – Additional 79 protein kinase cascade genes and 107 apoptosis-related genes follow the same pattern as the above 63 genes – 393 genes identified by Prediction Analysis of Microarray with R (PAM-R) 5 days after challenge as classifiers to distinguish protected, delayed onset of disease and non-protected groups	Pathways associated with protection: – Pre-challenge: proteasome degradation pathway (PSME2, PSMB9, PSMB6, PSMA4) up in the protected group – Post-challenge: apoptosis pathway Strong, transitory changes in genes associated with inflammatory response, apoptosis and protein kinase cascade	– Of the 39 vaccinees, 13 were protected and 26 were not – Results suggest a potential role of the vaccine in conferring MHC class I-mediated protection	Vahey *et al.* (2010)
Ad5hr-HIV/SIV (env, gag, nef) & HIV gp140 [recombinant, replicating adenovirus type 5 (host range) prime, recombinant protein boost]	Prime: 2× (i.n. & oral, followed by i.t.) Boost: 2× rec. protein i.m. 24 juvenile male rhesus monkeys Whole blood	– 2640 pre-challenged genes with |FC| > 2.0 and ANOVA $P < 0.01$ in > 4 animals in 3 treatment groups (i.e. Ad5hr-HIV/SIV with or without boost and a control Ad5hr vector alone without boost) – 1703 day 14 post-challenged genes with |FC| > 1.5, ANOVA $P < 0.01$ in > 4 animals – 551 genes week 12 post-challenged genes with |FC| > 1.5, ANOVA $P < 0.01$ in > 4 animals	Pathways associated with protection: – Pre-challenge: B-cell development (BST2, GPI, PDCD1, POUf2, MHCII, KEGG pathway for N-linked glycan analysis) and lymphocyte survival – Post-challenge at day 14: upregulated pathways include lymphocyte/leucocyte activation, mitochondrial process, also include IL27, complement components. Downregulated pathways include protein biosynthesis, protein folding, apoptosis, proteasome, mitochondrial dysfunction, Ag processing and presentation	– Significant pre-challenged and post-challenged (day 14/peak viraemia) signatures identified in protected animals – Expression profiles at set point (week 12 post-challenged) showed distinct immune responses despite similarities in viral load and CD4+ T cell levels (i.e. the boosted group showed evidence of Ab-dependent cell-mediated viral control	Palermo *et al.* (2011)

a. Influenza titre response index. The magnitude of HAI antibody response relative to other individuals in population, based on pre-and post-vaccination titres for all three antigens. |FC|, absolute fold change; FDR, false discovery rate.

BCMA belongs to a family of molecules (BAFF, APRIL, BAFF-R and TACI) that regulate the differentiation of plasma cells and antibody production. Notably, there were strong correlations between the expression of these genes and the magnitude of the HAI titres in response to vaccines against influenza and the magnitude of neutralizing antibody response to YF-17, which suggests that this network may be critically involved in regulating antibody responses to different vaccines (Nakaya *et al*., 2011). Thus, transcriptional profiling may provide an avenue towards identifying potential novel immune mechanisms that could be integrated into vaccine and adjuvant discovery and development (reviewed by Pulendran *et al*., 2010; Buonaguro and Pulendran, 2011).

Only a few human challenge models for infectious diseases exist, and thus far, genetic biomarkers predictive of vaccine efficacy have been described only for a recombinant malaria vaccine. In general, the value of vaccine immunoprofiling studies would be enhanced if more human challenge models for infectious diseases were developed.

Blood immune transcriptome analysis will also help answer two very important questions: why are the elderly less likely to develop protective immune responses after vaccination and why are there differences among individuals in terms of susceptibility to a particular infectious pathogen? To this end, the National Institute of Allergy and Infectious Diseases (NIAID) recently launched a 'Human Immune Phenotyping Initiative', a $100-million program to be carried out at six institutes across the USA to facilitate research that employs this immune 'fingerprints' approach to understand vaccines and infectious diseases. It can be anticipated that due to the increased availability of gene arrays and next-generation sequencing an increasing amount of genetic data linked to vaccine immunogenicity, safety and in some cases efficacy in humans will become available (Dhiman *et al*., 2009). It will, however, remain a challenge to identify true causal relationships between the genetic signature(s) induced by a specific vaccine and their observed biological correlates (Pulendran *et al*., 2010). In addition, comparison of genetic biomarkers predictive of safety and efficacy across different vaccines would benefit from some standardization of the methodologies applied to profiling and computational analysis.

With a few exceptions (e.g. BCMA), the set of affected genes (even for similar vaccine preparations, such as flu) seems to be rather large, heterogeneous and diverse, which currently limits their practical use as biomarkers. Furthermore, the comparability of studies is limited due to the variety of methods employed for detection and computation of gene expression differences and genetic signatures (Table 1); ultimate proof of the importance of certain genes may require their knock-out in animal models (Nakaya *et al*., 2011).

Nonetheless, these early studies of transcriptional profiling have provided remarkable insight into the vast panoply of genes involved in immune responses stimulated by vaccination. The challenge of the future is clear: how do we tease out of these data-specific markers that will predict safety and efficacy of vaccine candidates in development, thereby reducing the risks and costs of vaccine development.

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