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A case study

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Article type : Letter to the Editor

CD18 is redundant for the response to multiple vaccines: a case

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Running title: CD18 deficiency and multiple vaccinations

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To the Editor

A first born boy to unrelated healthy Danish parents was presented with marked neutro- and lymphocytosis after birth (Table 1). His umbilical cord did not separate until age five weeks. Due to chronic skin infection (without pus formation) and protracted leucocytosis (haematological malignancy was excluded) he was referred to our university hospital at the age of 11 months. We found elevated CD19⁺ B cell, CD4⁺ and CD8⁺ T cell concentrations (Table 1), normal concentrations of IgA, IgM and IgG (data not shown) but flow-cytometry revealed total absence (0%) of neutrophil expressed CD18 (integrin beta chain-2) consistent with severe leukocyte adhesion deficiency type 1 (LAD-1) . The functional relevance of his complete CD18 deficiency was confirmed by observing that his neutrophils failed to generate neutrophil extracellular traps (NETS) in response to *Staphylococcus aureus*¹, a process which depends upon functional MAC-1 and hence functional CD18. Microarray analysis and Sanger sequencing revealed a missense mutation (*ITGB2*: c.817G>A; pGly273Arg) on the paternal allele, known to prevent CD11a/CD18 hetero-dimerization² and a novel 1 Mb deletion (arr [hg19] 21q22.3 (45,441,009-46,511,411) encompassing the maternal *ITGB2* gene. Having received standard childhood multiple vaccinations at the age of three and five months respectively, our patient, now aged 12 months, received his third standard childhood multiple vaccination comprising: 1) *H. influenzae* type B conjugate, 2) (tridecavalent) pneumococcal conjugate vaccine (PCV), 3) toxoids (tetanus, diphtheria and pertussis) and 4) inactivated poliovirus. This standard regimen was supplemented with: 5) a live attenuated varicella zoster vaccine (VZV). The decision to include the VZV vaccine relied on the following considerations: 1) MMR infections being very rare in Denmark due to

reasonably high vaccination coverage, VZV infection was considered a greater risk for the patient. 2) Adequate antibody reactions were shown for the preceding immunizations indicating sound T-cell function; 3) Acyclovir was available in case of post-vaccination disease; 4) Parents received detailed instructions on how to observe any untoward events following vaccination.

Table 1 displays the PCV vaccination kinetics and the VZV, *H. influenzae*, *C. tetani* and *C. diphtheria* post-vaccination (21 days) titers (pre-transplantation determination of polio titers unfortunately failed due to technical reasons and the response to pertussis toxin is not used for assessing vaccination responses, sources: Statens Serum Institut, Denmark). At the age of 24 months (52 weeks post-vaccination), our patient received an allogeneic bone marrow transplantation. His infectious history until then was characterized solely by recurrent bacterial skin infections amenable to treatment with systemic antibiotics. Patient antibody levels remained protective 52 weeks post-vaccination except for antibodies against *H. influenzae* type B which had dropped below the long term protective level ($\geq 1\mu\text{g}/\text{mL}$) 20 weeks post-vaccination. In agreement with informed parental consent and with protocol: S-20150176 (The Regional Committees on Health Research Ethics for Southern Denmark) further immunological work-up was initiated. Patient $\text{CD4}^+\text{CD45RA}^-\text{CXCR5}^+\text{CCR7}^{\text{lo}}\text{PD-1}^{\text{hi}}$ peripheral T follicular helper cell (pT_{FH}) frequencies displayed the characteristic expansion 7 days post-vaccination (Table 1).

In-vitro, our patient's CD4^+ T cells were repeatedly non-responsive to plate bound anti-CD3/anti-CD28 (Table 1) as well as tetanus toxoid (data not shown). However, patient CD4^+ T cells repeatedly proliferated to stimulation with plate bound anti-CD3/anti-CD28/his-tagged (human) inducible T cell costimulatory ligand (ICOSL, Table1) (but not to plate bound

anti-CD3/anti-CD28/anti-his-antibody (data not shown)), expressed ICOS (Table 1), but produced less IL-2 (0.4 vs. control: 2.1 pg /mL) and IL-6 (266.3 vs. control: 3939.9 pg /mL) than control CD4⁺ T cells.

Patient CD4⁺ T cells proliferated, upregulating the T cell activation markers CD69 (57% of control level), CD25 (64% of control level) and ICOS (Table 1) in response to the TLR7 agonist R848 (1µg/mL) combined with IL-2 (10ng/mL). Concentrations of the GC chemokine CXCL13 as well as the cytokines IL-2, IL-6 and IL-33 peaked in R848 and IL-2 stimulated patient PBMC cultures sampled seven days post-vaccination (Figure 1). Except for IL-2, patient cytokine levels were approximately 2-3 fold (IL-33), 4-11 fold (IL-6) and >10 fold higher (CXCL13) than controls levels (seven days post-vaccination samples from three PCV and diphtheria-tetanus booster vaccinated healthy adult controls) (Figure 1). Three days of in-vitro stimulation with a combination of anti-CD3/anti-CD28 coated beads, which in itself constitutes a very potent stimulatory modality³, and IL-2 (10ng/mL) showed that seven days post-vaccination patient CD4⁺ T cells displayed more intracellular Bcl-6 (13.9% of CD4⁺ T cells) than those of a vaccinated control (5.2% of CD4⁺ T cells, data not shown) but only marginally more intracellular CXCL13 (9.8% of CD4⁺ T cells) compared to the vaccinated control (7.9% of CD4⁺ T cells, data not shown). Under steady state conditions and in response to anti-CD3/anti-CD28 stimulation, approximately 3% of peripheral CD4⁺ T cells are intracellular CXCL13⁺⁴.

LAD-1 is characterized by recurrent bacterial and fungal infections, without pus formation, despite leukocytosis. Clinical manifestations are caused by the inability of the integrin β2 chain (CD18) to form functional heterodimers with the CD11 family including CD11a with which CD18 forms the adhesion molecule lymphocyte function-associated antigen-1 (LFA-1).

Clinical severity depends on the degree of CD18 expression, as patients with < 1% of normal CD18 expression exhibit more severe infectious complications often leading to death in infancy if not transplanted. Data on vaccination outcomes in relation to complete human LFA-1 deficiency are sparse but unanimous and reveal quantitatively and qualitatively compromised antibody responses to primary as well as recall antigens^{5 6}. CD18 deficient mice additionally display impaired rejection of peripheral immunogenic tumors and show reduced lymphocyte homing to peripheral lymph nodes by 70-80%⁷. Collectively, this points to compromised lymphocyte lymph node homing/recirculation in CD18 deficient mice and men.

Despite elevated TCR thresholds and clinical indications of decreased vascular B and T cell adhesion, our patient developed protective antibody levels to all vaccines (constituting at least 18 different B cell receptor epitopes) within 21 days. Our case highlights the functional redundancy of human CD18 for a timely response to multiple protein vaccines, a fact consistent with a compensatory role of inflammation induced lymphocyte $\alpha 4$ integrin expression in compensating for the absence of CD18 during the process of lymphocyte lymph node homing.

For an extended characterization of our patient's immune response to multiple protein vaccines, we focused on his CD4⁺CD45RA⁻CXCR5⁺CCR7^{lo}PD-1^{hi} pT_{FH}. The expansion of this peripheral T cell subset, seven days post-vaccination, is concomitant with the formation of intra-germinal T follicular helper cell (T_{FH})⁸, the latter being crucial for the proper execution of B-cell responses to protein antigens⁹. Expansion of pT_{FH} is also dependent on the transcription repressor Bcl-6⁸. The increased levels of intracellular Bcl-6 protein, observed in

seven days post-vaccination patient CD4⁺ T cells, was consistent with his pronounced seven days pT_{FH} expansion.

As the master regulator of the germinal center (GC) reaction, controlling isotype switching and somatic hypermutation¹⁰, Bcl-6 provides the mechanistic link between pT_{FH} and high affinity antibody formation. Hence, ascertainment of pT_{FH} dynamics constitutes an accessible proxy marker for the evaluation of humoral responses to protein antigens.

The unresponsiveness to plate bound anti-CD3/anti-CD28 and recall antigens, displayed by our patient's T cells, contrasted with the unimpeded proliferation to these agonists normally displayed by CD18 deficient T cells^{5,11}. However, the T cell stimulatory effect which we obtained by adding immobilized ICOSL to the combination of anti-CD3/anti-CD28, suggested that ICOSL-ICOS stimulation effectively reduced our patient's elevated T cell activation thresholds consistent with the crucial role of ICOSL-ICOS interactions for intra-germinal T and B cell reactions¹².

Our patient's multiple vaccine response was congruous with robust production of the GC associated chemokine CXCL13, the latter being capable of regulating GC B and T cell interactions. The adjuvant effect of alum is partly mediated by the NALP3 inflammasome¹³.

As the TLR7 agonist R848 also activates the NALP3 inflammasome¹⁴, alum like pro-stimulatory mechanisms could account for the ability of R848 (+ IL-2) to exceed our patient's elevated CD4⁺ T cell activation thresholds. The interleukins IL-6 and IL-33 are generated in response to alum. IL-33 *per se* can induce caspase-1 independent production of IL-6, the latter, through the actions of STAT1 and STAT3, induces Bcl-6 expression⁹. Strong IL-2 signaling inhibits (through STAT5 induced Blimp-1 expression) Bcl-6¹⁵ and hence also pT_{FH} differentiation. Interestingly, despite the markedly elevated levels of CXCL13, IL-6 and IL-33,

which likely reflected the stimulatory effect of his multiple vaccinations, our patient's IL-2 level was not increased compared to three controls (Figure 1). This relative IL-2 deficiency was concordant with his elevated TCR thresholds and the reduced IL-2 production observed in CD18 deficient mice ¹¹. We suggest, based on our collective findings, a scenario where alum induced IL-33 and IL-6 combined with relatively reduced IL-2 production promoted strong Bcl-6 activity and hence facilitated a strong vaccine response. This case, which, to our knowledge, is the first of its kind, has clinical implications as it demonstrates a layer of inducible protective humoral immunity potentially attenuating some of the consequences of the compromised humoral and neutrophil immunity associated with severe LAD-1.

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Conflict of Interest or Acknowledgments

We have no conflicts of interest to declare.

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References Cited

1. Mohanty T, Sjogren J, Kahn F, et al. A novel mechanism for NETosis provides antimicrobial defense at the oral mucosa. *Blood*. 2015;126(18):2128-2137.
2. Hogg N, Stewart MP, Scarth SL, et al. A novel leukocyte adhesion deficiency caused by expressed but nonfunctional beta2 integrins Mac-1 and LFA-1. *J Clin Invest*. 1999;103(1):97-106.
3. Creson JR, Lin AA, Li Q, Broad DF, Roberts MR, Anderson SJ. The mode and duration of anti-CD28 costimulation determine resistance to infection by macrophage-tropic strains of human immunodeficiency virus type 1 in vitro. *J Virol*. 1999;73(11):9337-9347.
4. Kobayashi S, Watanabe T, Suzuki R, et al. TGF-beta induces the differentiation of human CXCL13-producing CD4(+) T cells. *Eur J Immunol*. 2016;46(2):360-371.
5. Fischer A, Durandy A, Sterkers G, Griscelli C. Role of the LFA-1 molecule in cellular interactions required for antibody production in humans. *J Immunol*. 1986;136(9):3198-3203.
6. Ochs HD, Nonoyama S, Zhu Q, Farrington M, Wedgwood RJ. Regulation of antibody responses: the role of complement and adhesion molecules. *Clin Immunol Immunopathol*. 1993;67(3 Pt 2):S33-40.
7. Berlin-Rufenach C, Otto F, Mathies M, et al. Lymphocyte migration in lymphocyte function-associated antigen (LFA)-1-deficient mice. *J Exp Med*. 1999;189(9):1467-1478.
8. He J, Tsai LM, Leong YA, et al. Circulating Precursor CCR7(lo)PD-1(hi) CXCR5(+) CD4(+) T Cells Indicate Tfh Cell Activity and Promote Antibody Responses upon Antigen Reexposure. *Immunity*. 2013;39(4):770-781.
9. Choi YS, Yang JA, Crotty S. Dynamic regulation of Bcl6 in follicular helper CD4 T (Tfh) cells. *Curr Opin Immunol*. 2013;25(3):366-372.
10. De Silva NS, Klein U. Dynamics of B cells in germinal centres. *Nat Rev Immunol*. 2015;15(3):137-148.
11. Varga G, Nippe N, Balkow S, et al. LFA-1 contributes to signal I of T-cell activation and to the production of T(h)1 cytokines. *J Invest Dermatol*. 2010;130(4):1005-1012.
12. Warnatz K, Bossaller L, Salzer U, et al. Human ICOS deficiency abrogates the germinal center reaction and provides a monogenic model for common variable immunodeficiency. *Blood*. 2006;107(8):3045-3052.
13. Kool M, Petrilli V, De ST, et al. Cutting edge: alum adjuvant stimulates inflammatory dendritic cells through activation of the NALP3 inflammasome. *J Immunol*. 2008;181(6):3755-3759.

14. Kanneganti TD, Ozoren N, Body-Malapel M, et al. Bacterial RNA and small antiviral compounds activate caspase-1 through cryopyrin/Nalp3. *Nature*. 2006;440(7081):233-236.
15. Ballesteros-Tato A, Leon B, Graf BA, et al. Interleukin-2 inhibits germinal center formation by limiting T follicular helper cell differentiation. *Immunity*. 2012;36(5):847-856.

Legends

Figure 1

PBMC cultures (1×10^6 /mL), sampled pre-, seven and 21 days post-vaccination, were stimulated for three days with IL-2 (10ng/mL) and R848 (1 μ g/mL). Cytokine measurements were performed using the Euroimmun CXCL13 ELISA kit (for CXCL13), Meso-Scale platform, V-plex plus human IL-2 kit, K151QQG-1 (for IL-2), and U-plex Biomarker Group 1, K15067L-1 (for IL-6 and IL-33). Analyses were performed according to the manufacturers instructions.

Table 1. Leukocyte, vaccination and 5 days stimulation characteristics

	(95%CI)*	LAD-1	Control ^{A)}
Neutrophils ($\times 10^9$ /L)	1.8–8.9	40.2	ND
CD4 ⁺ T cells ($\times 10^9$ /L)	0.7–2.5	7.6	ND
CD8 ⁺ T cells ($\times 10^9$ /L)	0.2–1.2	1.5	ND
CD19 ⁺ B cells ($\times 10^9$ /L)	0.3–1.1	4.2	ND
PCV pre-vaccination titers** (μ g/mL) (median)	ND	1.7	0.6
PCV titer (fold) changes (median)	ND	7.7	9.6
VZV post-vaccination titer (mIU/mL)**	ND	531	ND
<i>H. influenzae</i> b post-vaccination titer (μ g/mL)**	ND	1.4	ND
<i>C. diphtheria</i> toxin post-vaccination titer (IU/mL)**	ND	>26	13
<i>C. tetani</i> toxin post-vaccination titer (IU/mL)**	ND	10	17
CCR7 ^{lo} PD-1 ^{hi} P _{TH} (pre-/ 7/ 21 days) (% of CD4 ⁺ CD45RA ⁺ CXCR5 ⁺ T cells)	ND	15.0/22.8/21.3	13.6/19.7/14.4
PBMC (CD4 ⁺) *** (anti-CD3/anti-CD28)	ND	0.0	3.3
PBMC (CD4 ⁺) *** (anti-CD3/anti-CD28/ICOSL)	ND	3.5	3.4
ICOS (% of CD4 ⁺)(R848 + IL-2)	ND	3.0	3.2
ICOS (% of CD4 ⁺)(CD3/CD28/ICOSL)	ND	3.1	8.5

*In-house (age adjusted) normal range.

** Protective antibody levels: PCV: 0.35 μ g/mL, *H. influenzae* b: > 0.99 μ g/mL, *C. diphtheria* toxin: > 0.1 IU/mL, *C. tetani* toxin: > 0.02 IU/mL (Statens Serum Institut, Copenhagen, Denmark), VZV (varicella zoster vaccine): 150 mIU/mL (Liaison VZV IgG, DiaSorin S.p.A., Italy).

*** Proliferation index.

ND: Not determined, ^{A)}: Adult control.

Figure 1

