Identifying Central Components of the HIV-1+ Pregnancy Immune Network

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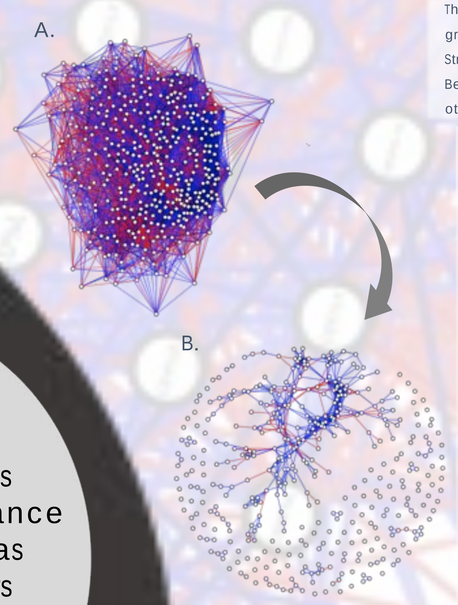
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- Successful pregnancy is reliant on the acceptance of a semi-allogeneic fetus[1]
- Systemic immune regulation is associated with maternal-fetal tolerance[1, 2]
- Frequency and activation of blood leukocytes change in complicated pregnancies[3-5]
- Higher preterm birth rates in women with HIV-1 despite successful ART[6-8]

Our work highlights shared immune components that may regulate tolerance of pregnancy, and has identified parameters uniquely impacted by HIV-1 that could negatively influence the pregnant immune network

The Question

What are the central components of the pregnant HIV-1 positive immune system in the peripheral blood and do they differ from pregnant women who are HIV-1 negative?



Figures A and B show correlation networks[9] generated for pregnant women who are HIV-1 negative, Figure A shows the uncorrected correlation network and Figure B shows the Benjamini and Hochberg corrected network[10]. The white dots represent individual parameters (nodes) and the lines connecting them show either positive or negative relationships (blue or red). The connections of these nodes were assessed and compared.

The below table shows the top 50 Strength and Betweenness centrality scores for the pregnant groups. These scores were calculated using the centrality analysis on the igraph R package[11, 12]. Strength describes the number and weight of the significant connections a node has, while Betweenness describes how frequently a node is passed through when the shortest paths between all other nodes is explored. Parameters in bold are present in the top 50 of both groups.

Strength				Betweenness			
HIV-1 negative pregnancy		HIV-1 positive pregnancy		HIV-1 negative pregnancy		HIV-1 positive pregnancy	
Values	Parameter	Values	Parameter	Values	Parameter	Values	Parameter
19.700143	CD38+HLA-DR+ CD8 EM	15.2694144	CD38 + CD8 Temra	9718	MFI CCR7 DC5	7302	NK % Lymph
18.3662628	PD-1+ CD4	13.5327127	MFI TIM-3 NK	7320	CD38+HLA-DR+ CD4 CM	7264	Cytolytic NK % Lymph
18.2221957	CD38+HLA-DR+ CD8 Temra	13.4901706	CD11b+ Cytolytic NK	6886	PD-1+ CD4 Ni	6744	CD11b+ Cytolytic NK
17.4429792	PD-1+ CD4 Temra	12.6004923	CD11b+ NK	6622	CD38+HLA-DR+ CD8 Temra	6058	MFI PD-1 CD4 RTE
17.2517792	PD-1+ CD4 Ni	11.8929433	PD-1+ CD4	6448	PD-1+ CD8	5272	CD4 % CD3+
16.9495547	CD38+HLA-DR+ CD8	11.7201165	PD-1+ CD4 EM	6300	CD4 CM c/L	4950	CD38 + CD8 Temra
16.7854786	CD38+HLA-DR+ CD4 CM	11.1981454	CD38 + CD4 RTE	6274	PD-1+TIM-3+ % CD4	4928	CD8 Tscm % Lymph
16.0882098	CD38+HLA-DR+ CD4 Temra	11.1911098	CD38 + CD4 Temra	6002	MFI CD86 DC5	4416	CD8 Tscm c/L
15.6421294	CD38 + CD4 Ni	11.022407	PD-1+ CD4 CM	5482	CD38+HLA-DR+ CD8 EM	4074	PD-1+TIM-3+ % CD8
15.5860429	PD-1+ CD4 CM	10.8633397	ncMo c/L	5062	CD38+HLA-DR+ CD8	3564	PD-1+ CD4
15.1130236	CD38+HLA-DR+ CD4	10.8164482	MFI PD-1 Th17	5048	CD4 EM c/L	3498	MFI CD16 CD4
14.3896712	CD38+HLA-DR+ CD4 EM	10.5777355	CD38+HLA-DR+ % Th17	4736	PD-1+ CD8 Temra	3200	Th17 % Lymph
14.2159386	CD38+HLA-DR+ CD8 RTE	10.3550384	CD38+ CD4	4634	PD-1+TIM-3+ % CD8	2914	Th17 % CD3+
14.171009	PD-1+ % iMo	10.2547607	CD38+HLA-DR+ CD8 RTE	4540	PD-1+ CD4 Temra	2340	PD-1+ CD4 CM
14.1211563	CD38+HLA-DR+ CD4 RTE	10.2270993	MFI CD28 CD8 Temra	4304	MFI PD-L1 NK	2282	PD-L1+ CD4 CM
13.9923324	PD-1+ CD4 EM	10.1633197	CD38+ CD8	4124	CD8 EM c/L	2204	PD-1+ CD8
13.9840183	CD38 + CD4 Temra	9.80437224	PD-1+TIM-3+ % CD8	3184	PD-L1+ % CD56–CD16+ NK	2160	PD-1+ CD4 Ni
13.8289631	CD38 + CD4 EM	9.51393594	MFI CD28 CD8 EM	3141	CD4 Tscm % CD4+	2074	CD38+HLA-DR+ CD4 EM
13.6467869	PD-1+ CD8	9.40336606	CD38 + CD4 EM	3046	TIM-3+ % CD8	1998	CD11b+ NK
13.2940885	CD38 + CD8 Temra	9.04699542	NK % Lymph	2670	CD38 + CD4 Ni	1936	PD-1+ % Cytolytic NK
12.9198556	CD4 EM c/L	9.01325535	PD-1+ % Th17	2436	TIM-3+ % Treg	1916	ncMo c/L
12.388226	CD38+ CD4	9.00680381	CD11b+ CD27+ CD56Bright NK	2430	CD28+ CD4 EM	1904	CD8 Ni c/L
12.3248122	CD38+ CD8	8.72327036	MFI CD28 CD4 Ni	2412	cDC1 % DC *corrected*	1890	CD38 + CD8 EM
12.2937479	CD38 + CD8 RTE	8.70925985	MFI CD28 CD4 EM	2147	CD4 Tscm % CD3+	1868	CD38+HLA-DR+ % Th17
12.1459689	CD38+HLA-DR+ CD4 Ni	8.70277251	MFI CD28 CD4 CM	2086	MFI CD28 CD4 EM	1768	CD38 + CD4 RTE
12.0508193	PD-1+ % cMo	8.69926988	MFI CD28 CD4 RTE	2020	MFI TIM-3 Cytolytic NK	1728	MFI CD38 CD8
12.009339	CD38 + CD4 CM	8.66071646	MFI CD28 CD8	2016	CD95+PD-L1+ % Cytolytic NK	1640	PD-L1+ CD8 EM
11.7972871	MFI CD28 CD4 RTE	8.54528906	MFI CD28 CD8 RTE	1932	CD8 CM c/L	1620	MFI PD-L1 CD8 RTE
11.4922879	CD38 + CD4 RTE	8.47888621	MFI CD28 CD4	1860	MFI TIM-3 NK	1548	MFI TIM-3 NK
11.4587553	PD-1+TIM-3+ % iMo	8.37600887	CD38+HLA-DR+ CD8 Temra	1782	CD38+HLA-DR+ CD4 EM	1470	CD11b+ CD27+ CD56–CD16+ NK
11.0018268	MFI CD28 CD4 Ni	8.22316131	PD-1+ CD8 EM	1766	PD-1+ % iMo	1438	CD38 + CD4 Temra
10.989579	MFI CD28 CD4	8.1369421	HLA-DR+ CD4 CM	1662	CD28+ CD8 Temra	1426	PD-1+ CD4 EM
10.9016412	MFI CD28 CD8	8.10733867	CD38 + CD8 RTE	1628	Treg c/L	1408	tMo c/L
10.5649884	MFI CD28 CD8 EM	8.06883404	CD11b+ CD56–CD16+ NK	1626	CD8– MAIT c/L	1380	CD4:CD8 Ratio
10.3987261	CD8 EM c/L	7.53477	CD38+HLA-DR+ CD8	1624	CD95+PD-L1+ % CD4	1334	PD-1+ % CD56Bright NK
10.3488709	Tc17 c/L	7.31269477	Cytolytic NK % Lymph	1456	CD4 Tscm % Lymph	1322	PD-L1+ CD4
10.3381103	CD38 + CD8 EM	7.29088853	HLA-DR+ CD4 EM	1440	PD-L1+ % iMo	1318	MFI CD38 CD4 Temra
10.3377615	CD8 CM c/L	6.42793555	HLA-DR+ CD8 Temra	1422	CD38+HLA-DR+ CD4 Ni	1298	CD8 Temra c/L
10.3035353	CD4 CM c/L	6.40538602	PD-L1+ CD8 EM	1412	MFI TIM-3 cMo	1256	Cytolytic NK % NK
10.2551686	PD-1+ CD8 Temra	6.33862937	PD-1+ CD8	1390	Th17 c/L	1238	PD-1+ % Th17
10.201787	MFI CCR7 DC5	6.27971353	PD-1+ CD4 Temra	1336	MFI CD38 CD8	1222	PD-1+ CD8 Temra
10.1583625	MFI CD28 CD4 Temra	6.24420655	CD38+HLA-DR+ CD4 EM	1328	CD8 c/L	1184	MFI CD40-L CD8
10.0450333	MFI CD28 CD4 CM	6.19170032	MFI PD-L1 CD8 RTE	1228	PD-L1+ CD4 CM	1158	CD11b+ CD27+ NK
9.98365237	MFI CD38 CD8	6.19032449	CD8 Ni c/L	1228	CD95+PD-L1+ % CD8	1138	CD40-L+ CD8 EM
9.94242777	MFI CD28 CD8 RTE	5.58127035	MFI PD-L1 CD8	1226	PD-1+ CD8 RTE	1112	PD-1+ CD4 RTE
9.72176701	CD8 c/L	5.50349058	tMo c/L	1224	H-Y - IL-2	1102	HLA-DR+ CD4 CM
9.55825827	HLA-DR+ CD4 Ni	5.43215528	CD11b+ CD27+ Cytolytic NK	1224	CD28+ CD8	1096	PD-1+ CD4 Temra
9.36562397	MFI CD28 CD4 EM	5.4095616	CD38+HLA-DR+ CD8 EM	1224	CD27+ CD56Bright NK	1056	PD-1+ CD8 RTE
8.90735351	CD40-L+ CD4 EM	5.40159926	PD-1+ CD4 Ni	1224	TIM-3+ % iMo	1002	PD-1+ % cMo
8.72493447	Th17 % Lymph	5.39054538	MFI PD-1 CD4 RTE	1224	MFI TIM-3 iMo	958	MFI CD38 CD4 RTE

Acronyms: median fluorescence intensity – MFI; recent thymic emigrants – RTE; naïve – Ni; stem cell memory like T-cell – Tscm; central memory – CM; effector memory – EM; terminally differentiated memory – Temra; calculated count per litre – c/L; mucosal associated invariant T cells – MAIT; regulatory T cells – Treg; IL-17 containing compartment – Th17/Tc17; dendritic cell – DC; classical monocytes – cMo; intermediate monocytes – iMo; non-classical monocytes – ncMo; total monocytes – tMo; natural killer cells – NK; CD56+CD16- NK – CD56bright; CD56+CD16+ NK – Cytolytic

Shared Central Immune Network Parameters:

- Strength measure: CD38+ T cells and CD38+HLA-DR+ co-expressing CD4 and CD8 subsets were identified, as well as PD-1+ cells, suggesting these activated T cells are influential in the pregnant immune system. MFI of CD28 on CD4 T-cell subsets also appeared in the top 50 of both cohort networks.
- Betweenness measure: CD38+HLA-DR+ CD4 T cells, PD-1+ CD4 and CD8 subsets and MFI of TIM-3 on NK cells were shared between groups.

The Dataset

- Freshly isolated peripheral blood mononuclear cells from uncomplicated ART treated HIV-1 positive pregnant (PP; n=21) and HIV-1 negative pregnant (NP; n=36) women were analysed using ELISpot assays and longitudinally validated flow cytometry
- Natural killer (NK) cells, monocytes (Mo), dendritic cells (DC), and both classical and non-classical T-cell subsets and surface markers were identified
- IFNγ, IL-2, IL-10 and granzyme B functional responses against Influenza, Epstein-Barr, Cytomegalovirus and the H-Y male antigen were assessed

The Analysis

- Individuals lacking functional or leukocyte subset data were excluded from analysis, as were parameters missing more than 25% data, and participants with under 90% complete datasets, leaving PP n=12 and NP n=14 for analysis. Remaining missing data was imputed as the group parameter median
- Non-parametric Spearman's correlation networks of the resulting 500+ parameter group datasets were generated, the Benjamini and Hochberg correction applied[10], and analysed to determine network centrality measures[11, 12]
- The top 50 Strength (number of significant associations adjusted by association strength) and Betweenness (times passed through in shortest paths between all other interacting parameters) centrality measures were compared[11]

Pregnant HIV-1 Positive Network Parameters:

- Strength measure: CD11b+ and CD27+ NK cells, NK frequency within lymphocytes, TIM-3 surface expression of NK cells, PD-L1+ CD8 T cells, counts of both tMo and ncMo cells, and CD38+HLA-DR+ and PD-1+ Th17 T cells.
- Betweenness measure: CD4 to CD8 T-cell ratio, NK subset frequencies in lymphocytes, CD38+HLA-DR+ and PD-1+ Th17 T cells plus Th17 frequency of T cells and lymphocytes, CD40-L+ CD8 EM subset, more PD-1+ NK and T-cell subsets.

Pregnant HIV-1 Negative Network Parameters:

- Strength measure: the CD40-L+ CD4 EM T-cell subset was observed as having a high Strength scores within their immune network.
- Betweenness measure: TIM-3+ Treg cells and Treg cells per litre, CD95+PD-L1+ co-expressing NK, CD4 and CD8 T cells, CD11b+ NK cells, cell surface expression of CCR7 and CD86 on the DC5 subsets, corrected frequency of the cDC1 subset within the DC compartment, CD8- MAIT cell count, and IL-2 response against the H-Y male antigen were all parameters in the top 50 for Betweenness score.

TIM-3 surface expression on NK cells was the only shared innate immune component found in the top 50, suggesting a role for this receptor during pregnancy[13]

Treg and dendritic cell parameters unique to HIV-1 negative pregnancies suggests these cells play an influential role in their immunological network[14, 15]

Shared adaptive immune components were predominantly associated with expression of activation and exhaustion markers instead of cell counts and frequencies

Parameters unique to HIV-1 positive pregnancies indicate that altered innate and adaptive cell counts and frequencies are relevant in shaping their immune networks[16, 17]

Our study shows that the pregnant immune network is altered in HIV-1 positive women, supporting studies that suggest the difference found in the incidence of pregnancy complications may not be due to ART alone[6]. Components identified in HIV-1 negative network correspond with populations that adapt to pregnancy (Treg and DC), indicating this analysis method identifies relevant parameters and promotes follow-up of central parameters found in the HIV-1 positive network[14, 15].

References

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