Critically Appraised Topic

Title: Immunogenicity assessment in whole blood vs. PBMC

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Clinical question (PICO): Does immunogenicity assessment of biological drugs (I) in whole blood assays provide advantages compared to PBMC assays (C) in human subjects ex vivo (P) as evaluated by several immunological readouts (O)

Clinical scenario

Search strategy human **AND** whole blood **AND** PBMC **AND** comparative study [ptype]

Search outcome Cochrane database – 1 [9]
TRIP database – 0
Pubmed – 76 relevant 14

1st Author	Year	Patients	Design	Assay	Result	Comment
Ashmore, L. M.	1989	3 Donors / 3 days	Experimental / comparative	FACS	Whole blood stain-lyse method of choice for the analysis of lymphocyte subsets in disease, particularly detection of activated T cells and if the whole blood samples would be stored overnight before preparation and staining.	
Suni, M. A.	1998	Donors	Experimental / comparative	Intracellular cytokine FACS analysis in stimulated samples	whole blood similar but slightly higher percentages of T cells responsive to specific antigen.	T cell responses to CMV in whole blood were observed only in sensitized individuals, and CD4 + T cell responses could be blocked by anti-class II MHC antibodies.
Fowke, K. R.	2000	4 Donors	Experimental / comparative	Viability / Apoptosis of cryopreserved samples	significant sample deterioration (viability, apoptosis, and function) in whole blood relative to PBMC	Whole blood requires prompt processing; not relevant for ex vivo testing of biologicals
Mayringer, I	2000	25 Donors	Experimental / comparative	TNF ELISA and RT-PCR	significant correlations between several methods for the determination of TNF-a production in white blood cells e.g., in vitro stimulation of whole blood and PBMC cultures and RT-PCR.	No qRT-PCR performed, accurate assessment of TNF-a production depends on the following factors: immediate processing of blood samples, appropriate anticoagulant and appropriate stimulant for whole-blood or PBMC stimulations.
Butscher, W. G.	2001	4 patients with autoimmune thyroid disease, three with AT, one with GD, and one normal individual	Experimental / comparative	IL-2–stimulated proliferation to Tg	Diluted whole blood is superior to the separated cell assay in detecting Tg-specific T-cell proliferation in autoimmune thyroid disease patients	
Schindler, R.	2001	Donors	Experimental / comparative	ELISA	LPS induced almost equal amounts of cytokines in whole blood and from PBMC, crude bacterial cultures induced much less cytokines in whole blood	Addition of erythrocytes but not granulocytes decreased cytokine induction by bacterial filtrates as well as by LPS, probably by adsorption of these substances. The addition of 30% plasma increased cytokine induction by LPS but decreased cytokine induction by P. aeruginosas filtrates.
Doherty, T. M.	2005	cohorts of TB patients, their household contacts and community controls	Multicentric	ELISA, ELISPOT, PCR	good agreement was obtained with the different IFN-g assays; ELISA and RT-PCR, results with whole blood were comparable to PBMC; sensitivity of the ELISpot assay was higher with PBMC	No qRT-PCR performed, PBMC may improve reproducibility but does not enhance sensitivity, under optimal conditions whole blood advantage
Maecker, H. T.	2005	Donors (3-6)	Multicentric (6-9 centers)	Intracellular cytokine FACS	Whole blood and cryopreserved PBMC	Shipped whole blood assays were also subject to data loss

				analysis in stimulated samples	showed grossly similar levels of reproducibility. However, when analysis variability was removed, cryopreserved PBMC processed with lyophilized reagents showed significantly better reproducibility than shipped whole blood.	when samples were not delivered in a timely fashion – not relevant for ex vivo testing
Pinto, L. A.	2005	20 HPV-vaccinated and 4 placebo treated recipients	Blinded HPV vaccination study	11 cytokines in multiplex bead arrays	highest levels of induction found for WB for several cytokines	multiplex assays for cytokine profiling in WB are an efficient tool for assessing broad spectrum, innate and adaptive immune responses to vaccines and identifying immunologic correlates of protection in efficacy studies
Appay, V.	2006	Healthy and vaccinated melanoma patients	Experimental / comparative	Facs-Analysis including tetramer staining of whole blood and PBMC	PBMC induce a bias in subpopulation distribution, in particular of CD8+ T cells, and lead to inaccurate measurement of antigen specific CD8+ T cell responses	use of whole blood for immuno-monitoring may be considered the method of choice, whenever possible and after verification that no pitfall precludes accurate assessment of markers of interest
Debey, S.	2006	6 male 6 female	Experimental / comparative	BeadChip oligonucleotide Array in PaxGene preserved samples with globin reduction	Globin reduction required to measure small changes in gene expression (male / female) in stabilized whole blood samples	No direct comparison on same samples
Silberer, J.	2008	25 adults 25 full term neonates	Experimental / comparative	IFN-g, IL-10, and IL-13 ELISA in supernatants of PHA and BLG stimulated whole blood , PBMC and CBMC	In adults, only levels of IL-10 were significantly correlated in WB and PBMC cultures. IFN-g and IL-13 levels in WB and CBMC/PBMC supernatants are not comparable.	standardized approach with exactly defined culture conditions is needed
Damsgaard, C.T	2009	64 healthy men	Experimental / comparative	IL-6, TNF and IL-10 ELISA in supernatants of LPS and L.acidophilus whole blood , PBMC and monocyte cultures	Cytokines produced from whole-blood was found to be more strongly correlated with monocytic cytokines	whole-blood cultures are well-suited low-cost proxy-measures of monocytic cytokine production. Moreover, large inter-individual variation in cytokine production was demonstrated whereas the individual responses in whole blood were reproducible even over long time-periods.
Chen, J.	2010	6 volunteers	Experimental / comparative	LPS induced TNF-a gene expression qRT-PCR	Higher expression in whole blood in comparison to fresh and frozen PBMC	fresh blood culture is a low-cost and easy setup approach which mimics the natural environment. Therefore, in clinical trials with large number of subjects, fresh whole blood could be the best choice for in vitro stimulation studies.

Comments

Advantages of whole blood assays: require small volumes of blood, basic laboratory facilities, are characterized by few preparation artifacts, deliver standardized performance, assessment of in vivo effects of drug therapies and immune therapies, measurement of vaccine efficacy, assessment of in vitro drug effects on the ability of T cells to mount a specific responses to antigens. Specific responses can be measured in the presence of autologous cellular and serum components that may be physiologically relevant.

Conclusion

Majority of analyzed manuscripts suggest, that whole blood assays are equally or superior in comparison to PBMC based assays. ELISPOT assays are superior in PBMC. Ex vivo testing should be performed in a whole blood scenario.

References

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