PRESERVATION OF FUNCTIONALITY, IMMUNOPHENOTYPE AND RECOVERY OF HIV RNA FROM PBMC'S CRYOPRESERVED FOR MORE THAN 20 YEARS.

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Background:
Repositories of cryopreserved peripheral blood mononuclear cells (PBMC) are costly to maintain but are of uncertain utility for immunological studies after decades of storage. We studied preservation of cell surface phenotypes and in-vitro function of PBMC after >20 years cryopreservation of samples from HIV+ versus healthy control subjects.

Methods:
Thawed PBMC were tested for viability and 18-colour flow cytometry immunophenotyping for major lymphocyte, monocyte and dendritic cell subsets, which was compared with the 1996 immunophenotyping of freshly collected blood. T cell function in thawed PBMC was assessed by polyclonal activation (anti-CD3/CD28/CD2), or response to influenza antigen, by measuring CD25/CD134(OX40) upregulation on CD4 T cells at day 2 of culture, and proliferative CD25+ CD4 blasts at day 7. Intracellular HIV RNA in extracted RNA from proliferating CD4+ blast cells was measured using short amplicons for both the Double R and pol regions by respective pi code assays, while, separately, long 4Kbp amplicons were sequenced up to and including pol.

Results:
Major PBMC sub-populations were well conserved. Proportions of naïve, memory and effector subsets of T cells in cryopreserved PBMC correlated with old results from the fresh blood, except for a decrease in activated CD38+HLA-DR+ CD4 and CD8 T cells from HIV+ PBMC samples. Polyclonal and antigen-specific T cell OX40 and proliferation responses were readily detected in cryopreserved PBMC from both HIV+ patients and healthy control donors. We were also able to activate production of HIV RNA from cultured PBMC after 27 years cryopreservation, detecting intracellular HIV-1 RNA transcripts. We also found long unspliced transcripts in both cells and supernatants from 5/12 donors, which were used to generate 4kb amplicons that mostly had ≥80% wild-type sequences, consistent with replication competence.

Conclusion:
This unique study provides strong rationale and validity of using well-maintained biorepositories to support immunovirological research even decades after collection.

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