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Defect in HSP90 expression in highly differentiated human CD8$^+$ T lymphocytes

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Dear Editor,

Repeated stimulation of T cells results in senescence and differentiation, which correlates with the loss of co-stimulatory molecules CD28 and CD27. Highly differentiated CD8$^+$CD28$^-$CD27$^-$ T cells can display functional defects, and their increase during ageing may explain the decreased efficiency of the immune system in older humans.[1] CD8$^+$ T cells can also be separated on the expression of CD45RA and CD27 molecules into four populations, each showing progressive features of differentiation and ageing (Figure 1a). Highly differentiated effector memory (EM) and EM re-expressing CD45RA (EMRA) CD8$^+$ T cells are largely CD28$^-$CD27$^-$, and accumulate in older humans and in patients with persistent viral infections and inflammatory syndromes such as rheumatoid arthritis.[1,2]

CD28$^-$CD27$^-$ T cells show the shortest telomeres and lack of telomerase activity, impaired IL-2 production and proliferation that correlates with a defective AKT signalling controlled by the inhibitory receptor KLRG1 and other inhibitory molecules.[3] Indeed AKT controls CD8$^+$ T-cell growth and gene expression determining memory *versus* effector CD8$^+$ T-cell fate and trafficking patterns.[4] We here examine the molecular changes relevant to the defective AKT pathway that we have previously observed in highly differentiated CD8$^+$ T cells.[1,3]

HSP90 is a molecular chaperone essential for the stability of nascent client proteins involved in signal pathways controlling cell physiology. Impairment of HSP90 in T cells has been shown to block proliferation, IL-2 secretion and IL-2 receptor expression.[5] It is also essential for the correct assembly of the telomerase holoenzyme and for the interaction of the catalytic subunit hTERT with AKT, critical for telomerase activation.[6]

We observed a gradual decline of RICTOR (an essential component of mTORC2 complex responsible for AKT phosphorylation at Ser473) and HSP90 as CD8$^+$ T cells progress towards terminal differentiation (Figure 1b). A reduced interaction of AKT with either RICTOR or HSP90 was observed in highly differentiated CD28$^-$CD27$^-$ CD8$^+$ T cells as a result of the reduced expression of both the mTORC2 component and the chaperone (Figure 1b). This contributes to explain the reduced activation of AKT observed in highly differentiated T cells. Interaction between activated AKT, hTERT and HSP90 is required for hTERT phosphorylation and its nuclear import.[6] Also, such interaction was reduced in CD28$^-$CD27$^-$ T cells (Figure 1b). AKT is highly dependent on HSP90 for stability and to prevent proteasome-mediated degradation.[7] Absence of phosphorylation by mTORC2 makes the decreased levels of HSP90 observed in highly differentiated T cells even more critical for AKT function and stability. This role was demonstrated by blocking HSP90 with geldanamycin in undifferentiated CD8$^+$CD28$^+$ T cells, which resulted in the decline of AKT activation and the impairment of hTERT stability and its nuclear import in this subset (Figure 1b). Reduced HSP90 and pSer473-AKT were also found in highly differentiated CD8$^+$ T cells separated by CD45RA and CD27-based isolation (Figure 1c).

Our results show for the first time a defect in HSP90 expression in highly differentiated CD8$^+$ T cells, thus providing new insights about the possible critical role of the chaperone in the defective functionality of senescent T cells. The decline of HSP90 may contribute to the reduced proliferation, IL-2 production and telomerase activity of these cells. HSP90 impairment has recently been associated with the loss of CD28 molecule in lymphocytes.[8] Therefore the loss of HSP90 can directly contribute to the shaping of a differentiated phenotype in CD8$^+$ T cells. The identification of biochemical changes in end-stage differentiated CD8$^+$ T cells can provide new molecular targets in order to preserve the replicative capacity and functionality of CD8$^+$ T cells in old individuals and in models of immune senescence.

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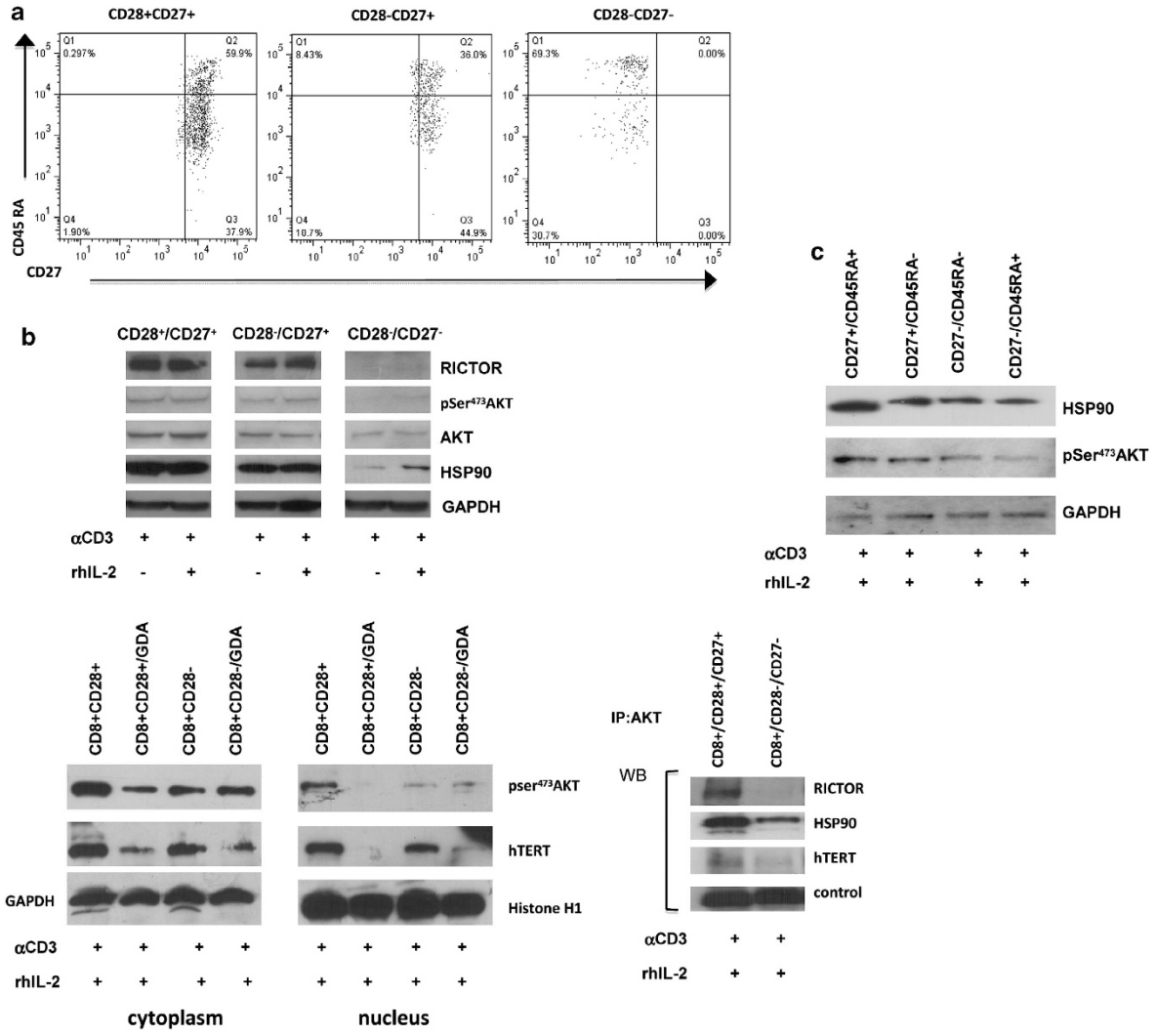

Figure 1 Signalling defects in highly differentiated CD8$^+$ T cells. Buffy Coat samples were obtained from the Transfusion Center of University of Rome 'Tor Vergata'. T-cell subsets were isolated according to the expression of the co-stimulatory molecules CD28 and CD27 using Miltenyi Biotec microbeads (Auburn, CA, USA) and activated with anti-CD3 antibody, Ab (purified OKT3 0.5 μg/ml), in the presence or absence of rhIL-2 (5 ng/ml, R&D systems, Minneapolis, MN, USA) at 37 °C in a humidified 5% CO_2 incubator. CD45RA/27-based populations were obtained by labeling CD8$^+$ T cells with conjugated anti-CD27 and CD45RA Abs (BD Biosciences, San Jose, CA, USA) and sorting populations using a FACSAria (BD Biosciences). This separation gives four populations with progressive features in terms of differentiation and ageing, with naive cells being CD27$^+$/CD45RA$^+$, central memory CD27$^+$/CD45RA$^-$, effector memory CD27$^-$/CD45RA$^-$ and highly differentiated effector memory (EM)-like phenotype (EMRA) that re-express the CD45RA molecule. Abs used for western blot analysis were from Cell Signalling (Danvers, MA, USA) and Santa Cruz Biotechnology (Dallas, TX, USA). The quality of extracts and loading control was tested by probing membranes with anti-GAPDH and anti-Histone H1 Abs for cytoplasmic and nuclear extracts, respectively. Cytoplasmic extracts were negative for Histone H1 (data not shown). For co-immunoprecipitation analysis HNGT-cell lysates were immunoprecipitated with anti-AKT1 Ab, separated by SDS-PAGE, and probed with RICTOR, HSP90 and hTERT Abs. (**a**) Representative dot plots of the phenotypic analysis of CD28/27 and CD45RA/27 CD8$^+$ T-cell populations. (**b**) To achieve full activation, AKT has to be phosphorylated at Ser473 by mammalian target of rapamycin (mTOR) complex 2 (mTORC2). CD28$^-$/27$^-$ subpopulation showed impaired RICTOR-AKT signaling activation and HSP90 expression partially reversed by rhIL-2. This result was confirmed by reduced levels of AKT-co-immunoprecipitated RICTOR, HSP90 and hTERT. Improper function of HSP90 client proteins can be induced by the HSP90 inhibitor geldanamycin (100 nM), which reduced pSer473-AKT and hTERT nuclear import in naive CD28$^+$ T cells without affecting T-cell viability. (**c**) Reduced HSP90 and pSer473-AKT levels were found also in highly differentiated CD8$^+$ T cells separated by CD45 RA and CD27-based isolation

Conflict of Interest

The authors declare no conflict of interest.

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1. Plunkett FJ *et al. J Immunol* 2007; **178**: 7710–7719.
2. Henson SM, Riddell NE, Akbar AN. *Curr Opin Immunol* 2012; **24**: 476–481.
3. Henson SM *et al. Blood* 2009; **113**: 6619–6628.
4. Kim EH *et al. J Immunol* 2012; **188**: 4305–4314.
5. Kim EH *et al. Cell Stress Chaperones* 2000; **5**: 52–61.
6. Kawauchi K, Ihjima K, Yamada O. *J Immunol* 2005; **274**: 5261–5269.
7. Facchinetti V *et al. EMBO J* 2008; **27**: 1932–1943.
8. Bae J *et al. J Immunol* 2013; **190**: 1360–1371.