HIV-1 modulates macrophage apoptosis in response to *S. pneumoniae* infection

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Abstract

Invasive pneumococcal disease including pneumonia is more common in those with HIV-1 infection. An elevated risk remains despite antiretroviral therapy and CD4 recovery, implicating HIV-1 in disrupting immune defence against S. pneumoniae (Spn) beyond T cell mediated immunity. Timely macrophage (Mφ) apoptosis is critical to the early resolution of Spn infection in the lung. Mφ from HIV-1seropositive individuals exhibit prolonged survival and resistance to apoptosis. We investigated whether HIV-1 infection alters the Mφ apoptotic response to Spn. Methods: 3 *in vitro* models of alveolar Mφ +/- HIV infection were used; the pro-monocytic cell line U937 vs. its HIV-1 expressing clonal derivative U1; healthy donor monocyte derived Mφ (MDM) +/- HIV-1 gp120 and MDM from HIV-1 viraemic individuals/controls. Each was challenged with opsonised type 2 Spn (D39) or mock infection (MI) and apoptosis measured over 20 hours by counting apoptotic nuclei (microscopy), hypodiploid DNA content (flowcytometry) and caspase-3/7 activity. Results: After differentiation, more U1 (26.1% ±4.73) contained hypodiploid DNA than U937 (16.9% ±2.55, n=6). The increment in hypodiploid DNA containing cells following Spn exposure was significantly smaller for U1 than U937 (p=0.008). Similarly, fewer U1 (24.2% ±11.40) than U937 (53.7% ±14.5, n=4) developed apoptotic nuclei and caspase 3/7 activity increased in U937 but not in U1 post Spn. 14 day old MDM, challenged with Spn, in the presence of gp120, were less likely to develop apoptotic nuclei and had smaller increments in caspase 3/7 activity than when challenged with Spn alone. Compared with HIV-1-seronegative controls, MDM from HIV-1 viraemic subjects had a smaller increment in the hypodiploid DNA population following Spn. Conclusion: Following Spn challenge, HIV-1 infection is associated with reduced induction of Mφ apoptosis, and gp120 may be sufficient to mediate this. HIV-1 infection may result in a Mφ phenotype that resists apoptosis in response to Spn. This could undermine the bactericidal and anti-inflammatory advantage gained by Mφ apoptosis following Spn phagocytosis and may contribute to susceptibility to pneumococcal disease in HIV-1-seropositive individuals.

Introduction & Background

- **Elevated risk of invasive pneumococcal disease and pneumonia persists among HIV-1 seropositive individuals in the HAART era [1,2]**
- **Alveolar macrophages play a pivotal role in preventing subclinical pneumococcal (Spn) infection developing into pneumonia and bacteraemia [3]**
- **After Spn internalisation a delayed programme of macrophage (Mϕ) apoptosis**
  - **Augments bacterial killing [4]**
  - **Limits damaging pro-inflammatory signalling [5]**
  - **Is dependent on dynamic changes in anti-apoptotic Mcl-1 [6]**
- **Persistence of HIV-1 in Mϕ is associated with a virus induced pro-survival phenotype mediated by viral proteins including HIV-1 gp120 and tat [7,8]**
- **We investigate whether these effects of HIV-1 impact on Mϕ responses to Spn**

Methods & Results

Spn infection

Cells infected/mock infected (MI) with opsonised, log phase D39 (type 2 Spn), MOI 10:1

Measurement of Apoptosis

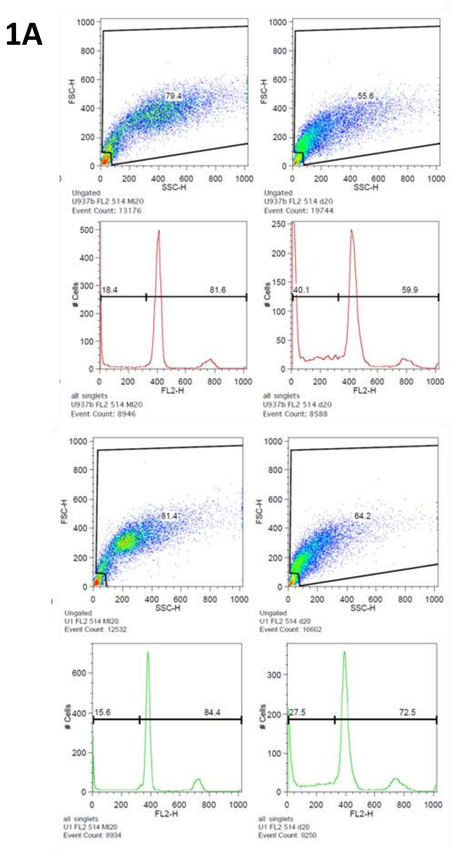
Hypodiploid DNA content (sub G0/1 population on FACS cell cycle analysis); Caspase 3/7 activation with fluorimetric assay [9]; Counts of cells with DAPI stained nuclei showing morphological changes of apoptosis or TUNEL positivity [10]

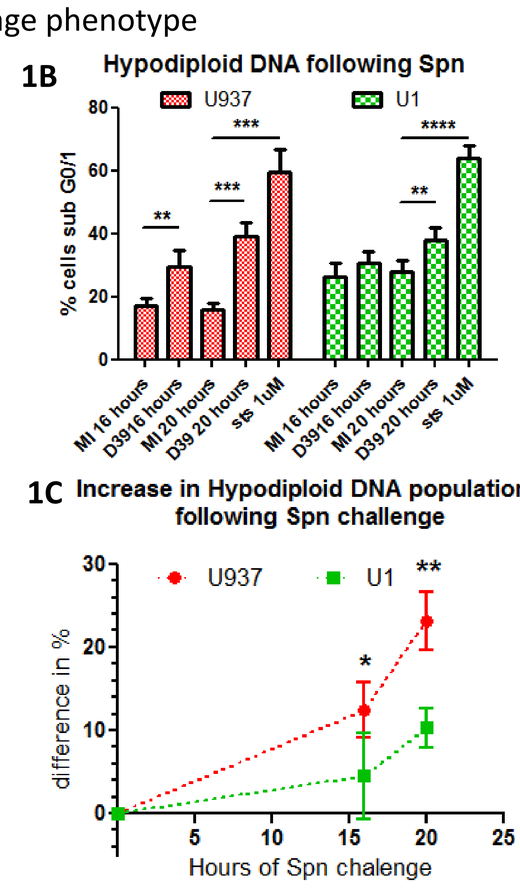
Internalisation & Killing Assay

MDM were washed at 4 hr postinfection, incubated with 20 µg/ml gentamicin for 30 min at 37°C, washed, and then lysed (and viable counts performed of internalised viable bacteria) or incubated with 0.75 µg/ml vancomycin until lysed at 20 hr

Model 1 : PMA differentiated U1 and U937

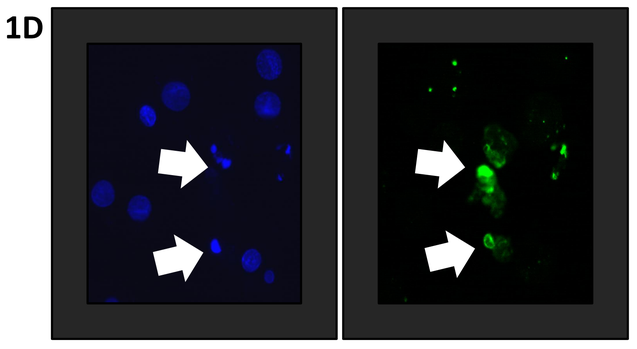
• Promonocytic cell lines U937 (control) and U1 (U937 latently infected with HIV-1) [11] treated with 100nM PMA for 2-3days then rested for 3-5days to activate HIV-1 replication and differentiate cells towards macrophage phenotype

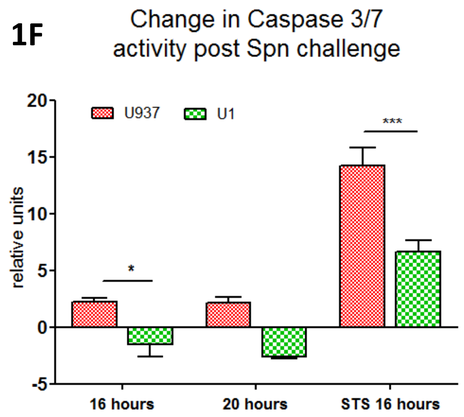




* p<0.05, ** p<0.01, *** p<0.005, **** p<0.001, paired t test

1A Representative dot plot illustrating gating for sub G0/1 population on cell cycle analysis
1B Larger hypodiploid DNA containing populations were measured in U1 than U937 with MI (n=8, p<0.05).There were increases in hypodiploid DNA cell populations following d39 or staurosporine (sts) (n≥6).
1C There were greater increases in hypodiploid DNA containing populations for U937 than U1 after 16 (n=6) and 20 hours d39 (n=8)



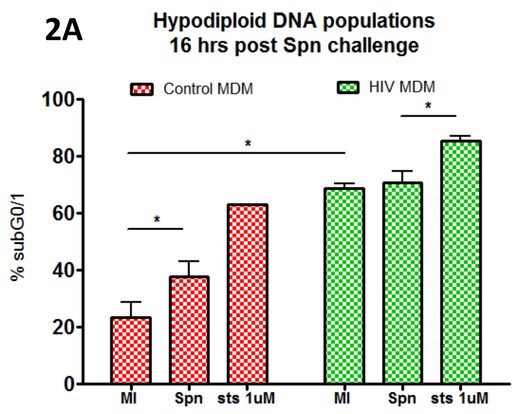


1D representative micrographs showing DAPI (blue) and TUNEL +ve (green) apoptotic nuclei
1E 20 hours after d39 there was a significant and larger increase in apoptotic U937 than U1 cells (n=4)
1F The relative increase in caspase 3/7 following Spn (or 1µM staurosporine) was significantly greater in U937 than U1 cells (n≥4)

* p<0.05,*** p<0.005, paired t test

Model 2 : MDM from HIV-1 viraemic individuals

•14 day monocyte derived macrophages from healthy but viraemic, ART naive subjects or HIV-1 seronegative controls

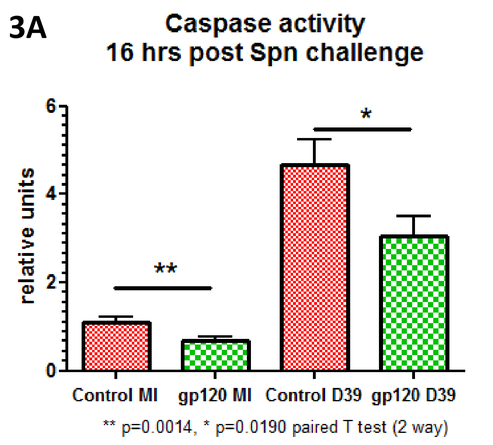


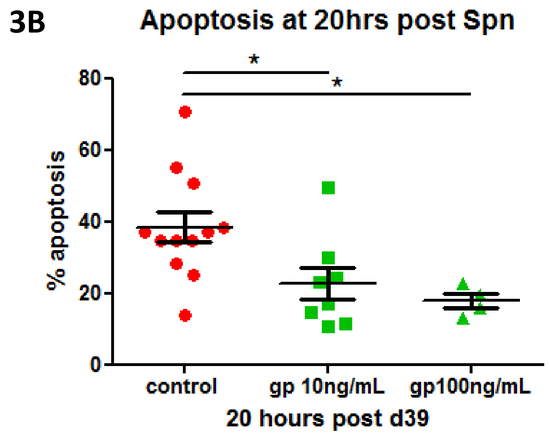
2A Significantly more MDM from HIV-1 donors had hypodiploid DNA than controls following MI.(n≥3)
U937, but not U1, had a significant increase in hypodiploid DNA content with Spn

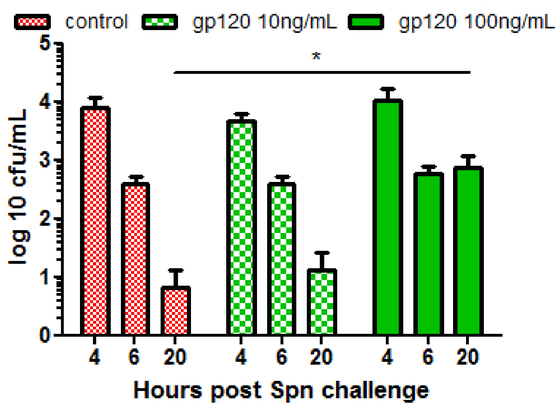
* p<0.05 unpaired t test

Model 3 : HIV-1 seronegative donor MDM treated with gp120

•14 day monocyte derived macrophages from HIV-1 seronegative donors, treated with 10ng – 100ng/mL gp120 [12] at start of Spn challenge







3A Significantly smaller increases in caspase 3/7 activity were detected in MDM co-treated with HIV-1 gp120 than control following Spn (n=5) **3B** The proportions of apoptotic nuclei were significantly smaller with both 10ng/mL and 100ng/mL gp120 co-treatment v control (p=0.0118, One way ANOVA with Bonferroni's post test) **3C R**ates of bacterial internalisation at 4 hours were similar but bacterial survival was significantly greater in gp120 treated MDM than control (p<0.0293, One way ANOVA with Bonferroni's post test, n≥4)

Conclusions

- **In all 3 models, HIV-1 is associated with reduced macrophage apoptosis in response to challenge with pneumococci**
- **HIV-1 gp120 may be sufficient to mediate this effect alone**
- **Bacterial internalisation is unaffected but there is impaired intracellular killing of Spn in macrophages exposed to HIV-1 gp120, associated with reduced apoptosis**
- **Monocytes infected with HIV-1 and subsequently differentiated into macrophages have increased constitutive apoptosis compared with controls**

References: 1. Jordano Q Clin Infect Dis. 2004 Jun 1;38(11):1623-8; 2. Grau I Arch Intern Med. 2005 Jul 11;165(13):1533-40; 3. Blumenthal RL J Allergy Clin Immunol. 2001 Feb;107(2):258-64; 4. Dockrell DH J Immunol. 2003 Nov 15;171(10):5380-8; 5. Marriott HM J Immunol. 2006 Nov 1;177(9):6480-8; 6.Marriott HM J Clin Invest. 2005 Feb;115(2):359-68; 7. Swingler S PLoS Pathog. 2007 Sep 7;3(9):1281-90; 8. Chugh P Retrovirology. 2008;5:11; 9. Sensolyte™ Homogenous AMC Caspase 3/7 assay kit, Anaspec 10. Apoptag in situ Direct kit, Milipore 11. AIDS Research and Reference Reagent Program, Division of AIDS, NIAID, NIH 12. recombinant HIV-1 IIIB envelope glycoprotein gp120 Programme EVA Centre for AIDS Reagents, NIBSC, HPA, UK