3rd Portuguese-Spanish Biophysics Congress

ALTERATION OF LYMPHOCYTE AND ERYTHROCYTE MEMBRANE PROPERTIES IN HIV-1 INFECTED PATIENTS

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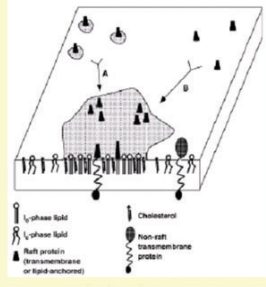
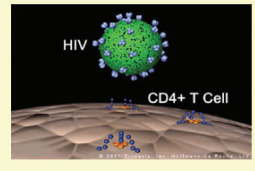
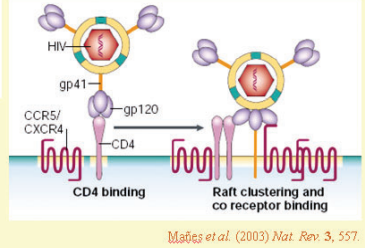
In order to evaluate the possible HIV-1 infection-induced changes on cell membrane properties and on calcium signaling, membrane fluidity, acetylcholinesterase (AChE, a glycosylphosphatidylinositol-anchored protein) activity and intracellular calcium concentration ($[Ca^{2+}]_{int}$) were evaluated in lymphocytes and erythrocytes of infected individuals, previously to their engagement in antiretroviral therapy. Membrane fluidity was assessed by measuring the fluorescence anisotropy of the membrane probes diphenylhexatriene (DPH) and trimethylamino-diphenylhexatriene (TMA-DPH). When compared with the control group, lymphocytes of infected patients presented significantly decreased membrane fluidity, decreased AChE activity and increased $[Ca^{2+}]_{int}$. Erythrocytes from HIV-infected patients presented decreased $[Ca^{2+}]_{int}$, when compared with the control group, and decreased membrane fluidity near the lipid / water interface. Our data

shows that HIV-1 infection leads to biochemical and biophysical changes on the membrane itself and on a membrane protein activity in lymphocytes and even in erythrocytes of HIV-infected patients. The present observations are consistent with a process of facilitated propagation of the infection to new cells, stimulation of virion production and maintenance of a reservoir of erythrocyte-bound infectious virus.

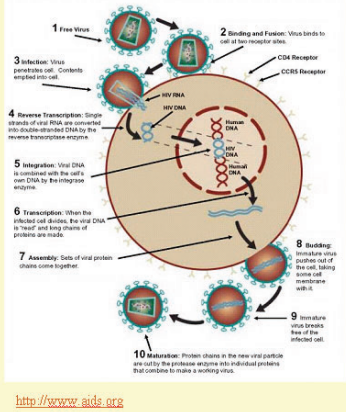


Brown & London (2000) *JBC* **275**, 17221.





Mañes *et al.* (2003) *Nat. Rev.* **3**, 557.



http://www.aids.org

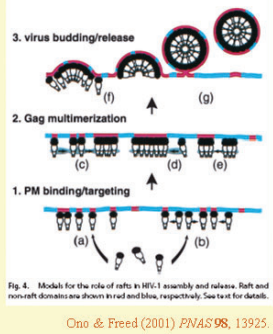
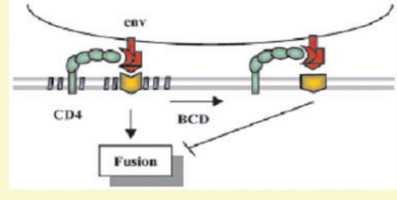
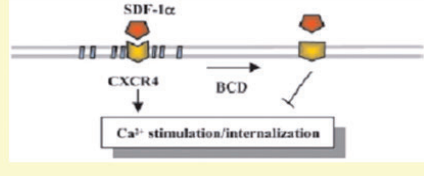


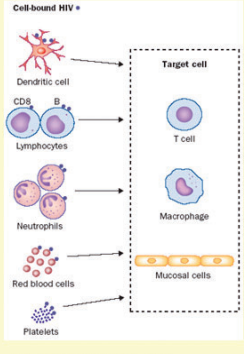
Fig. 4. Models for the role of rafts in HIV-1 assembly and release. Raft and non-raft domains are shown in red and blue, respectively. See text for details.

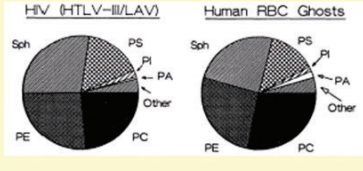
Ono & Freed (2001) *PNAS* **98**, 13925.





Nguyen & Taub (2002) *J. Immunol* **168**, 4121.





Levy (2002) *Lancet* **359**, 2212.

Hess *et al.* (2002) *Lancet* **359**, 2230.

Alois *et al.* (1988) *PNAS* **85**, 900.

Objective

To evaluate possible HIV-1 infection-induced changes on:

- membrane fluidity
- AChE activity (a GPI-anchored protein)
- $[Ca^{2+}]_{int}$

both in:

- lymphocytes
- erythrocytes

of male and female infected patients (previously to engagement in anti-retroviral therapy).

Samples

Infected patients (Serviço de Infecciologia, Santa Maria Hospital):

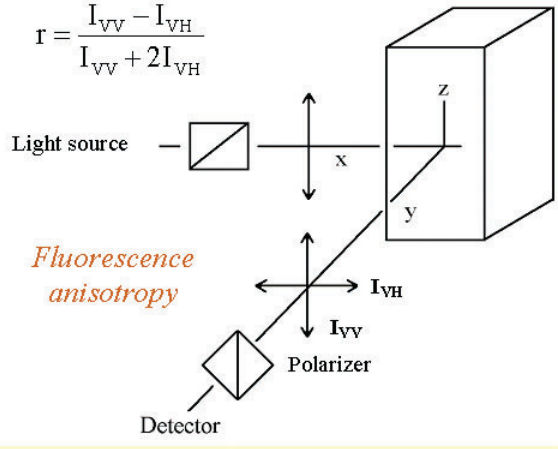
- N = 45
- 26 male
- 19 female

Controls (Portuguese Blood Institute):

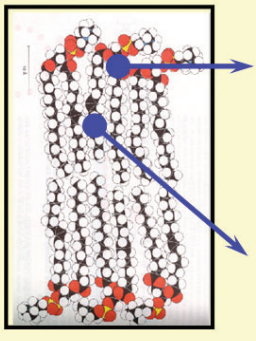
- N = 55
- 29 male
- 26 female

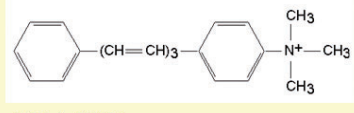
Methods

$$r = \frac{I_{VV} - I_{VH}}{I_{VV} + 2I_{VH}}$$

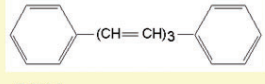


Methods





TMA-DPH



DPH

Methods

AChE activity → Ellman's method

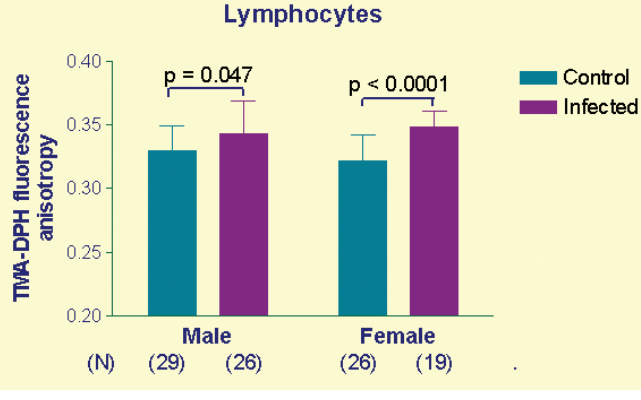
Intracellular Ca^{2+} → Fluorescence spectroscopy (fura-2)

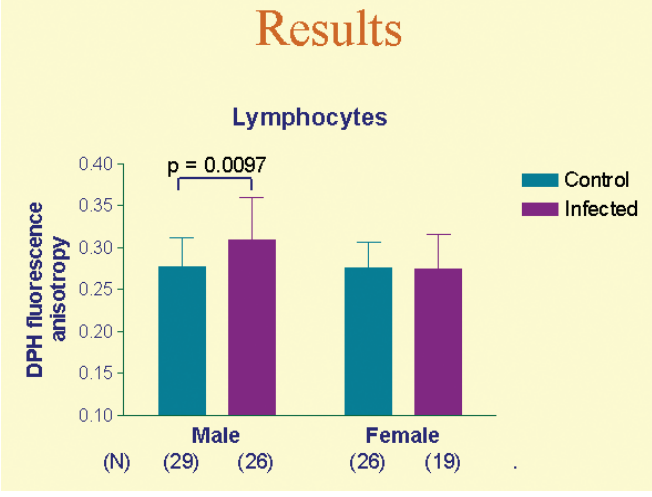
Results

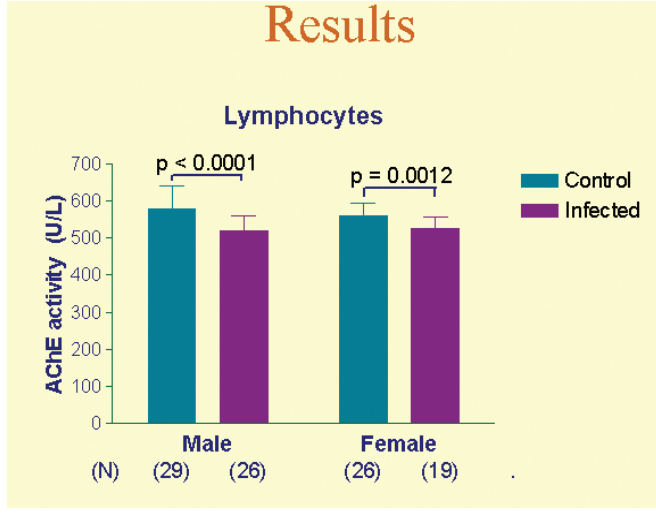
Immunologic and hematologic parameters of HIV-infected patients

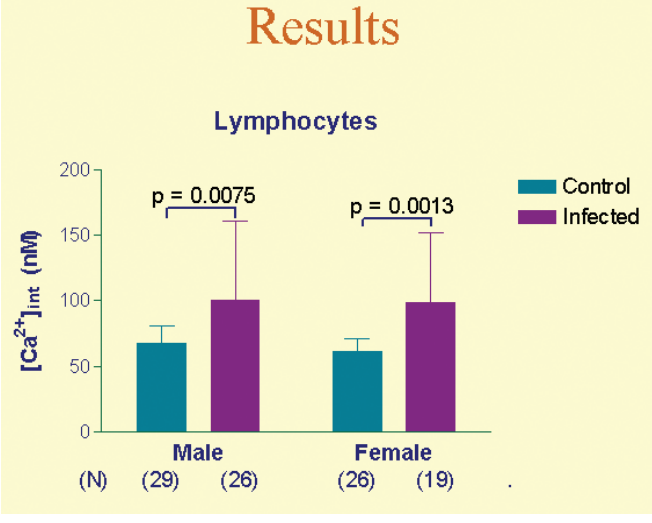
	Male (N = 26)	Female (N = 19)
[Hemoglobin], g/dL	8.8 – 16.0	10.8 – 14.8
Hematocrit, %	26.2 – 47.4	32.0 – 44.0
Leukocyte count, /mm^3	2600 – 11900	3000 – 8700
CD3$^+$ count, /mm^3	518 – 2938	456 – 3059
CD4$^+$ count, /mm^3	8 – 1951	19 – 1279
CD8$^+$ count, /mm^3	163 – 1813	350 – 2773
Viral load, /mL	43 – 5144838	240 – 269111

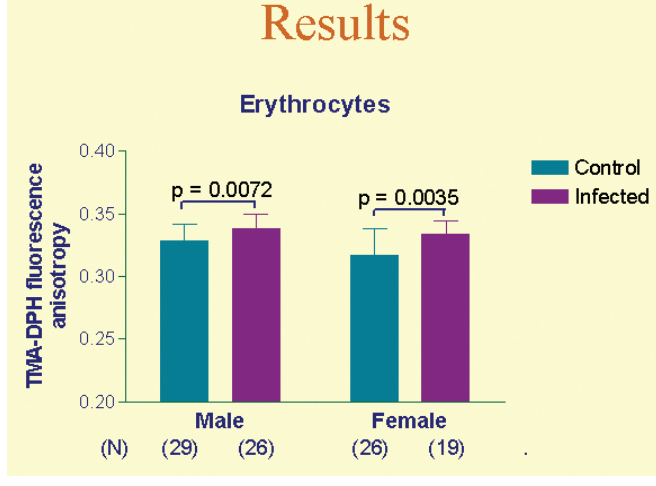
Results

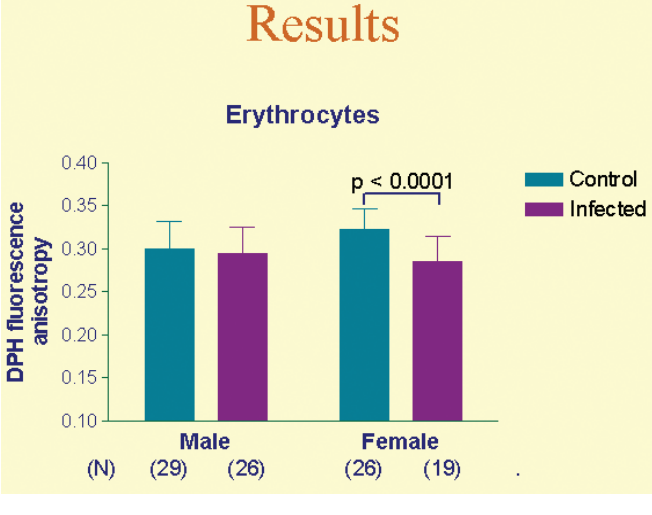


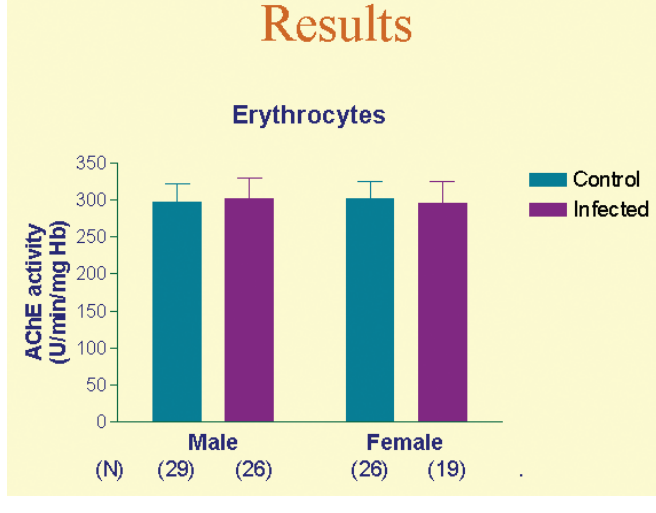

Results
Lymphocytes
DPH fluorescence anisotropy
0.40
0.35
0.30
0.25
0.20
0.15
0.10
p = 0.0097
Control
Infected
Male
Female
(N) (29) (26) (26) (19)

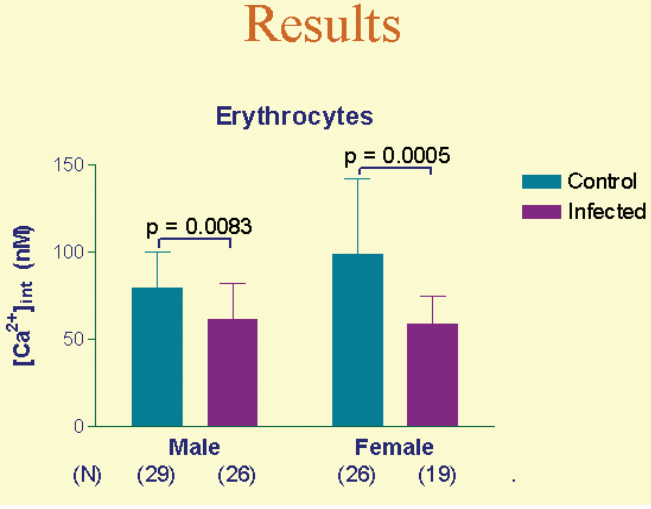

Results
Lymphocytes
AChE activity (U/L)
700
600
500
400
300
200
100
0
p < 0.0001
p = 0.0012
Control
Infected
Male
Female
(N) (29) (26) (26) (19)

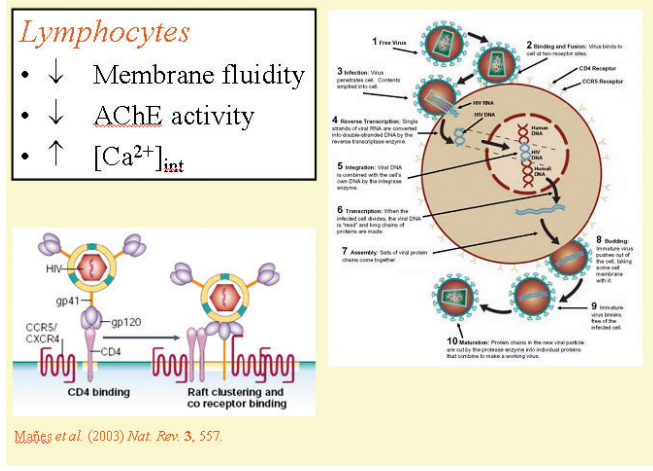

Results
Lymphocytes
[Ca2+]int (nM)
200
150
100
50
0
p = 0.0075
p = 0.0013
Control
Infected
Male
Female
(N) (29) (26) (26) (19)

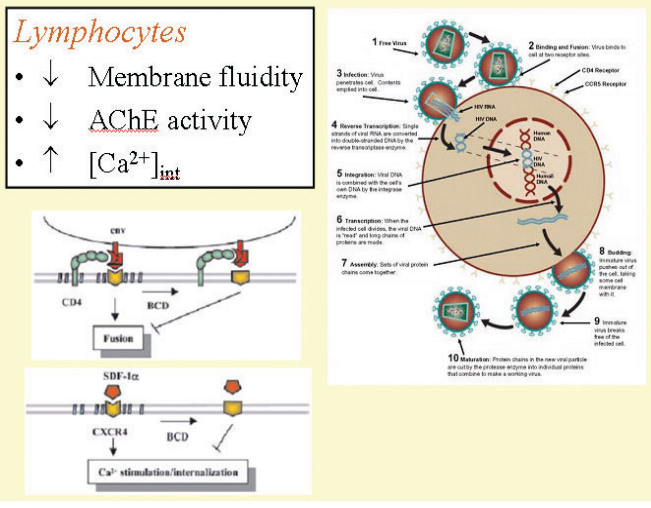

Results
Erythrocytes
TMA-DPH fluorescence anisotropy
0.40
0.35
0.30
0.25
0.20
p = 0.0072
p = 0.0035
Control
Infected
Male
Female
(N) (29) (26) (26) (19)

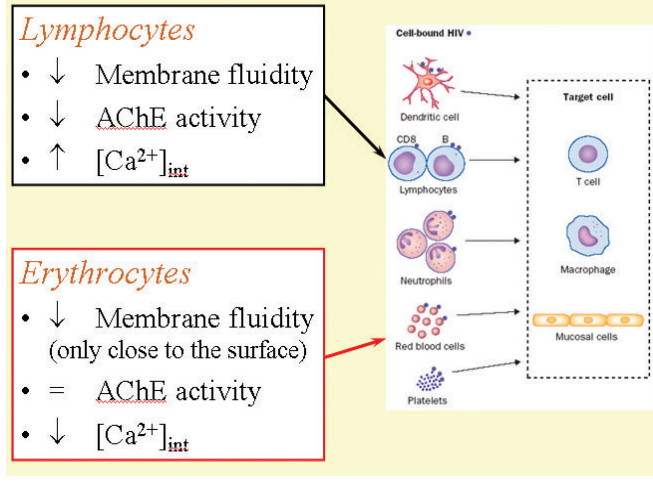

Results
Erythrocytes
DPH fluorescence anisotropy
0.40
0.35
0.30
0.25
0.20
0.15
0.10
p < 0.0001
Control
Infected
Male
Female
(N) (29) (26) (26) (19)


Results
Erythrocytes
AChE activity (U/min/mg Hb)
350
300
250
200
150
100
50
0
Control
Infected
Male
Female
(N) (29) (26) (26) (19)


Results
Erythrocytes
p = 0.0005
p = 0.0083
Control
Infected
$[Ca^{2+}]_{int}$ (nM)
150
100
50
0
Male
Female
(N) (29) (26) (26) (19)


Lymphocytes
• ↓ Membrane fluidity
• ↓ AChE activity
• ↑ $[Ca^{2+}]_{int}$
HIV
gp41
gp120
CCR5/
CXCR4
CD4
CD4 binding
Raft clustering and
co receptor binding
Mañes et al. (2003) Nat. Rev. 3, 557.


Lymphocytes
• ↓ Membrane fluidity
• ↓ AChE activity
• ↑ $[Ca^{2+}]_{int}$
CD4
BCD
Fusion
SDF-1α
CXCR4
BCD
Ca2+ stimulation/internalization


Lymphocytes
• ↓ Membrane fluidity
• ↓ AChE activity
• ↑ $[Ca^{2+}]_{int}$
Erythrocytes
• ↓ Membrane fluidity
(only close to the surface)
• = AChE activity
• ↓ $[Ca^{2+}]_{int}$
Cell-bound HIV
Dendritic cell
CD8
B
Lymphocytes
Neutrophils
Red blood cells
Platelets
Target cell
T cell
Macrophage
Mucosal cells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