Impaired antigen presentation to CD4+ T-cells by HIV-infected monocytes is related to down-modulation of CD4 expression on helper T-cells: Possible involvement of HIV-induced cellular factors

Audrey T. Louie[b], Larry M. Wahl[c], Indira K. Hewlett[b], Jay S. Epstein[b], Subhash Dhawan[b,a,*]

[a] *Laboratory of Immunochemistry, Division of Transfusion Transmitted Diseases, 1401 Rockville Pike (HFM-320), Rockville, MD 20852-1448, USA*
[b] *Imunopathogenesis Section, Laboratory of Molecular Virology, Division of Transfusion Transmitted Diseases, Center for Biologics Evaluation and Research, Food and Drug Administration, Bethesda, MD, USA*
[c] *Laboratory of Immunology, National Institute of Dental Research, Bethesda, MD, USA*

Received 9 September 1996

Abstract Defective antigen presentation by HIV-infected monocytes is related to severe immune dysfunction in patients with AIDS, although the mechanism by which this process occurs is not well defined. Here we report that reduced capacity by HIV-infected monocytes to stimulate or present antigen to CD4+ T-cells was mediated by cellular factors associated with the plasma membranes of HIV-infected monocytes. In contrast, soluble factors secreted by HIV-infected monocytes had little or no effect on T-cell stimulation. Reduced T-cell stimulation by HIV-infected monocytes was related to down-modulation of CD4 expression on helper T-cells and was not affected by the inclusion of anti-HIV-gp120 Ab, indicating the involvement of soluble or cell-associated viral envelope protein to be less likely. Exposure of CD4+ T-cells, that had been in co-culture with HIV-infected monocytes, to uninfected monocytes partially restored impaired T-cell stimulation. Thus, for the first time we report that altered capacity of HIV-infected monocytes to stimulate and present antigen to CD4+ T-cells is related to down-modulation of CD4 expression on T-cells, and appears to occur via membrane-associated cellular factors on HIV-infected monocytes.

Key words: HIV; AIDS; Monocyte; T-cell; Antigen presentation

1. Introduction

Monocytes play an important role in the pathogenesis of HIV infection [1–3]. These cells serve as virus reservoir in affected tissues, vectors for virus transmission to the central nervous system, and potent source of inflammatory cytokines which can affect tissue function and virus replication [4,5]. In addition to being major target cells for HIV infection, monocytes also play a critical role as antigen presenting cells which allows antigen recognition and proliferation of CD4+ T-cells [6]. Previous studies on peripheral blood monocytes from HIV-infected patients and in vitro have demonstrated defects in antigen presentation by monocytes [7–11]. Although humoral responses have been reported to be normal, the ability to respond to antigens is diminished with the progression of the disease [12,13]. The basis for unresponsiveness of CD4+ T-cells to antigen which may contribute to dysregulation of immune function seen during the course of HIV disease is, therefore, important to study. In the present study, we have examined the involvement of viral and cellular components associated with HIV-infected monocytes on the ability of these cells to stimulate and present antigen to CD4+ T-cells. We have demonstrated that altered accessory function of HIV-infected monocytes is associated with down-modulation of CD4 expression on helper T-cells and is mediated, at least in part, by cell membrane-associated factors.

2. Materials and methods

2.1. Isolation and HIV infection of monocytes

Monocytes were purified from PBMC of donors seronegative for HIV and hepatitis B infection after leukapheresis and purified by countercurrent centrifugal elutriation [14]. Cell suspensions contained >95% monocytes by criteria of cell morphology on Wright-stained cytosmears, by granular peroxidase, and by non-specific esterase. After 5–7 days of culture, monocytes in 96-well plastic plates, were exposed to HIV-1_{ADA}, a monocytotropic HIV strain (Advanced Biotechnologies Inc., Columbia, MD) at the desired multiplicity of infection (MOI), infectious virus/target cell [15]. After a 2 h viral adsorption interval, cultures were washed and refed with fresh medium. Culture medium was half-exchanged with fresh medium every 2–3 days.

2.2. Preparation of monocyte membranes

Membranes from monocytes were prepared according to the method of Lin et al. [16]. All steps were carried out at 4°C unless otherwise indicated. Briefly, cultured, uninfected and HIV-infected monocytes were washed three times with PBS, resuspended in 50 mM mannitol (Sigma Chemical Co., St. Louis, MO) containing 5 mM HEPES, and a cocktail of protease inhibitors (consisting of 1 mM PMSF, 10 μM pepstatin A, 10 μM antipain, 10 μM leupeptin, and 10 μM chymostatin), pH 7.4, and sonicated to disrupt cells. $CaCl_2$ was added to a final concentration of 10 mM and incubated at 4°C for 20 min with gentle rotation. The homogenate was centrifuged at $5000 \times g$ for 10 min to remove nuclei and calcium-induced membrane aggregates. The supernatant containing plasmalemma vesicles was further centrifuged for 1 h at $48\,000 \times g$ to isolate plasma membrane in an ultracentrifuge (Beckman Instruments). The translucent membrane pellet was washed twice with PBS, and resuspended in DMEM by triturating with a 1 ml syringe in polypropylene tube freeze vials and stored under nitrogen at −80°C until used.

2.3. Preparation of CD4+ T-cell population

Total T-cell population was obtained by elutriation [14]. Residual accessory cells were removed by plastic adherence for monocytes followed by panning on anti-Ig-coated plastic plates for B-cells. This T-cell enriched population was used to further purify CD4+ T-cells by depleting CD8+ T-cells using columns immobilized with anti-CD8 mAb according to the protocol suggested by the manufacturer (Pierce

*Corresponding author. Fax: (1) (301) 594-6989.
E-mail: dhawan@a1.cber.fda.gov

Abbreviations: MOI, multiplicity of infection; MLR, mixed leukocyte reaction; HIV, human immunodeficiency virus; AIDS, acquired immunodeficiency syndrome; TCR, T-cell receptor; Ag, antigen.

PII S0014-5793(96)01164-7

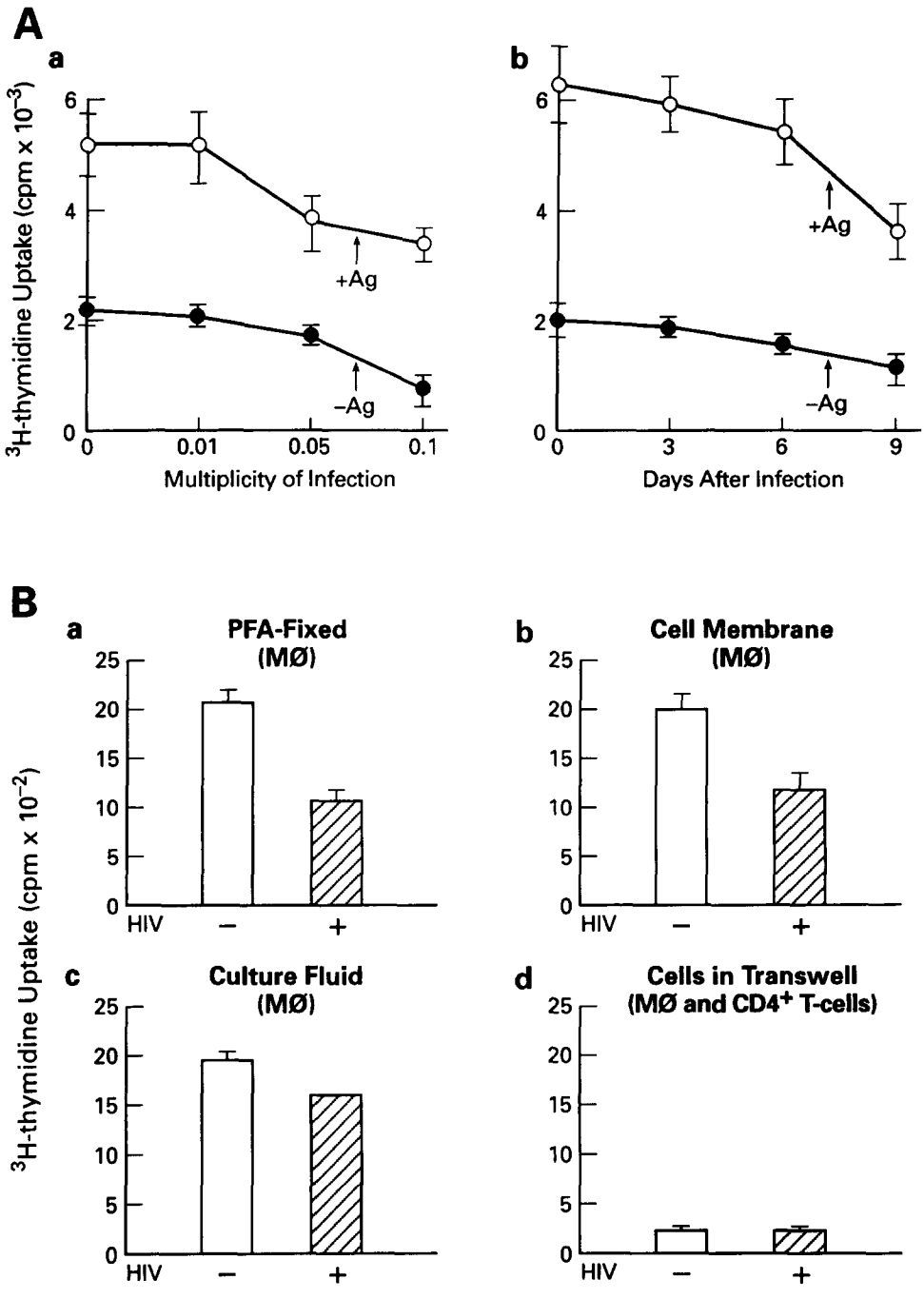

Fig. 1. Effect of HIV infection on monocyte-induced T-cell stimulation and antigen presentation to CD4+ T-cells. (A) Freshly isolated CD4+ T-cells (10^6 cells/well) were added to monocytes, and incubated at 37°C for 72 h. T-cell proliferation was determined by [^{3}H]thymidine incorporation as described in Section 2. (a) T-cell stimulation by monocytes infected with HIV at various MOI; (b) T-cell stimulation by monocytes infected with HIV at various times after infection. (○) +antigen; (●) −antigen. (B) (a) Uninfected or HIV-infected monocytes were fixed with 4% paraformaldehyde for 2 h, washed with PBS, and incubated with CD4+ T-cells; (b) CD4+ T-cells were incubated with plasma membranes from uninfected (open bar) or HIV-infected monocytes (hatched bar); (c) T-cell stimulation by monocytes in the presence of culture fluid from uninfected (open bar) or HIV-infected monocytes (hatched bar); (d) stimulation by uninfected (open bar) or HIV-infected monocytes (hatched bar) of T-cells separated by a transwell system. The data are representative of three experiments.

Chemical Co., Rockford, IL). The CD4+ enriched T-cell population (>98%) thus obtained was used for T-cell proliferation assays.

2.4. T-cell proliferation assay

Uninfected and HIV-infected monocytes (10^5 cells/0.2 ml) were incubated in the presence or absence of tetanus toxoid (500 ng/ml) for 2 h, washed with DMEM, and then were co-incubated with CD4+ T-cells (10^6 cells/well) for 3 days. T-cell proliferation was measured by the uptake of [^{3}H]thymidine (20 Ci/mM, New England Nuclear, Boston, MA) which was added (1 μCi/well) on the third day of culture. Cells were harvested 16 h later using a Millipore multiscreen vacuum manifold with type C glass fiber filter plates (Millipore, Bedford, MA) and incorporated radioactivity was measured using a liquid scintillation counter.

2.5. Detection of CD4 and CD8 expression on T-cells by flow cytometry

CD4 or CD8 expression on T-cell population was determined by

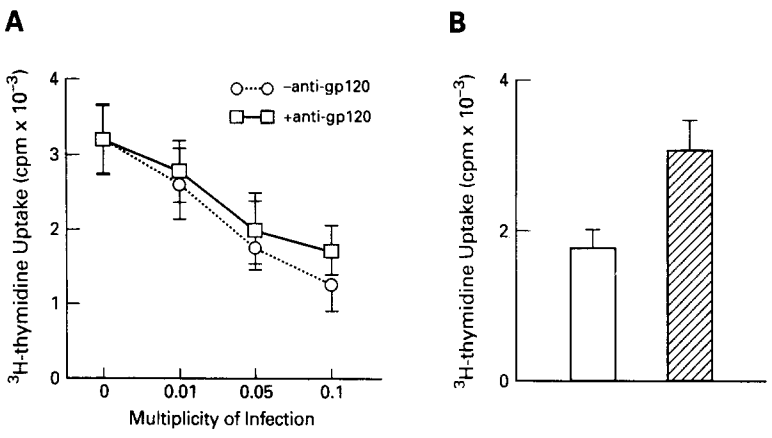

Fig. 2. Effect of anti-gp120 Ab on monocyte-induced T-cell stimulation. (A) Monocytes infected with HIV at various MOI were incubated with CD4+ T-cells in the presence or absence of polyclonal anti-gp120 Ab (50 μg/ml), and T-cell proliferation was determined as described in Section 2. (B) Recovery of reduced T-cell proliferation by uninfected monocytes. CD4+ T-cells in co-culture with HIV-infected monocytes for 3 days were added to uninfected monocytes, incubated for 3 additional days, and T-cell proliferation was determined as described in Section 2. Open bar, stimulation by HIV-infected monocytes of T-cells that had been in co-culture with HIV-infected monocytes for 3 days; hatched bar, stimulation by uninfected monocytes of T-cells that had been in co-culture with HIV-infected monocytes for 3 days.

flow cytometry according to a protocol described earlier for p24 detection [17] with slight modifications. Briefly, purified T-cells, total T-cells or PBMC co-cultured with uninfected or HIV-infected monocytes after 3 days of incubation were recovered, washed twice with PBS, and resuspended in binding buffer (PBS containing 2% FBS, 0.2% BSA, 0.2% NaN_3, and 1 mg/ml human immunoglobulins). Cells were then incubated with CD3-FITC/CD4-PE or CD3-FITC/CD8-PE mAb (Becton Dickinson, Mountain View, CA) for 30 min at 4°C. Cells were then washed twice with cold PBS, fixed in 4% paraformaldehyde, and examined by flow cytometry (Becton Dickinson).

3. Results

The ability of monocytes after HIV infection to stimulate and present antigen to CD4+ T-cells was examined by a mixed leukocyte reaction (MLR) assay. Monocytes were infected with HIV at various MOI, and co-incubated with CD4+ T-cells in the present or absence of tetanus toxoid (500 ng/ml) for 3 days. T-cell stimulation was determined by [^{3}H]thymidine incorporation in T-cells. HIV-infected monocytes at various MOI resulted in a significant reduction of T-cell proliferation as shown in Fig. 1A. Consistently, HIV infection of monocytes also reduced their ability to present antigen to CD4+ T-cells (Fig. 1A,a). Similar results were obtained when CD4+ T-cells were co-incubated with HIV-infected monocytes at various times after infection (Fig. 1A,b). Thus, the reduced capacity of HIV-infected monocytes to stimulate CD4+ T-cells was directly related to viral load in the infected monocytes. Reminiscent with the findings of Fig. 1A, HIV-infected monocytes after fixation with 4% paraformaldehyde as well as cell membranes from infected cells also demonstrated reduced T-cell proliferation by more than 2-fold (Fig. 1B; a and b, respectively). In contrast, cell-free culture fluid from uninfected or HIV-infected monocytes had no effect on monocyte-induced T-cell proliferation (Fig. 1B,c). Similarly, no induction of T-cell proliferation was observed when monocytes and T-cells were separated by a 0.3 μm pore size polycarbonate filter in a transwell system (Fig. 1B,d), suggesting cell to cell contact to be critical for T-cell stimulation.

To determine whether reduced T-cell proliferation by HIV-infected monocytes was mediated by gp120 which is expressed on infected cells [18], HIV-infected monocytes were incubated with CD4+ T-cells in the presence or absence of polyclonal anti-gp120 Ab and T-cell proliferation was assayed. The inclusion of excessive amounts of polyclonal anti-gp120 Ab did not influence impaired T-cell stimulation (Fig. 2A), indicating reduced T-cell stimulation by HIV-infected monocytes was a process independent of CD4-gp120 interactions. Furthermore, addition of soluble viral proteins HIV-gp120 or HIV-Tat had no effect on resting T-cell proliferation (not shown). Thus, the involvement of HIV proteins in reduced T-cell proliferation appears less likely. To determine the ability of uninfected monocytes to restore impaired T-cell function, T-cells that had been in co-culture with HIV-infected monocytes, were exposed to uninfected monocyte cultures, and the ability of these uninfected monocytes to stimulate treated T-cells was assessed after 72 h. The results from these experiments shown in Fig. 2B clearly indicate a partial recovery in T-cell stimulation by uninfected monocytes. These results suggest the impaired T-cell stimulation caused by HIV-infected monocytes to be a reversible phenomenon.

CD4 plays an important role in monocyte-mediated T-cell stimulation [19]. To elucidate whether the reduced T-cell stimulation by HIV-infected monocytes was related to altered regulation of CD4 expression on T-cells, purified CD4+ T-cells were co-cultured with uninfected and HIV-infected monocytes for 72 h, and the levels of CD4 were determined by flow cytometry. T-cells co-cultured with HIV-infected monocytes exhibited down-regulation of CD4 expression (>2-fold, Fig. 3a) as compared to those cultured with control uninfected monocytes. Similar results were obtained when total T-cell population or PBMC were co-incubated with HIV-infected monocytes (Fig. 3b and d, respectively). No significant effect on CD8 expression was observed (Fig. 3c and e, respectively). Also, no differential surface HLA-DR expression was observed between uninfected and HIV-infected monocytes (not shown).

To explain a possible mechanism involved in reduced T-cell proliferation by HIV-infected monocytes, we have proposed a working model depicted in Fig. 4. As shown in this model, HIV infection may result in the induction of host factor(s) on the surface of infected monocytes. These membrane-asso-

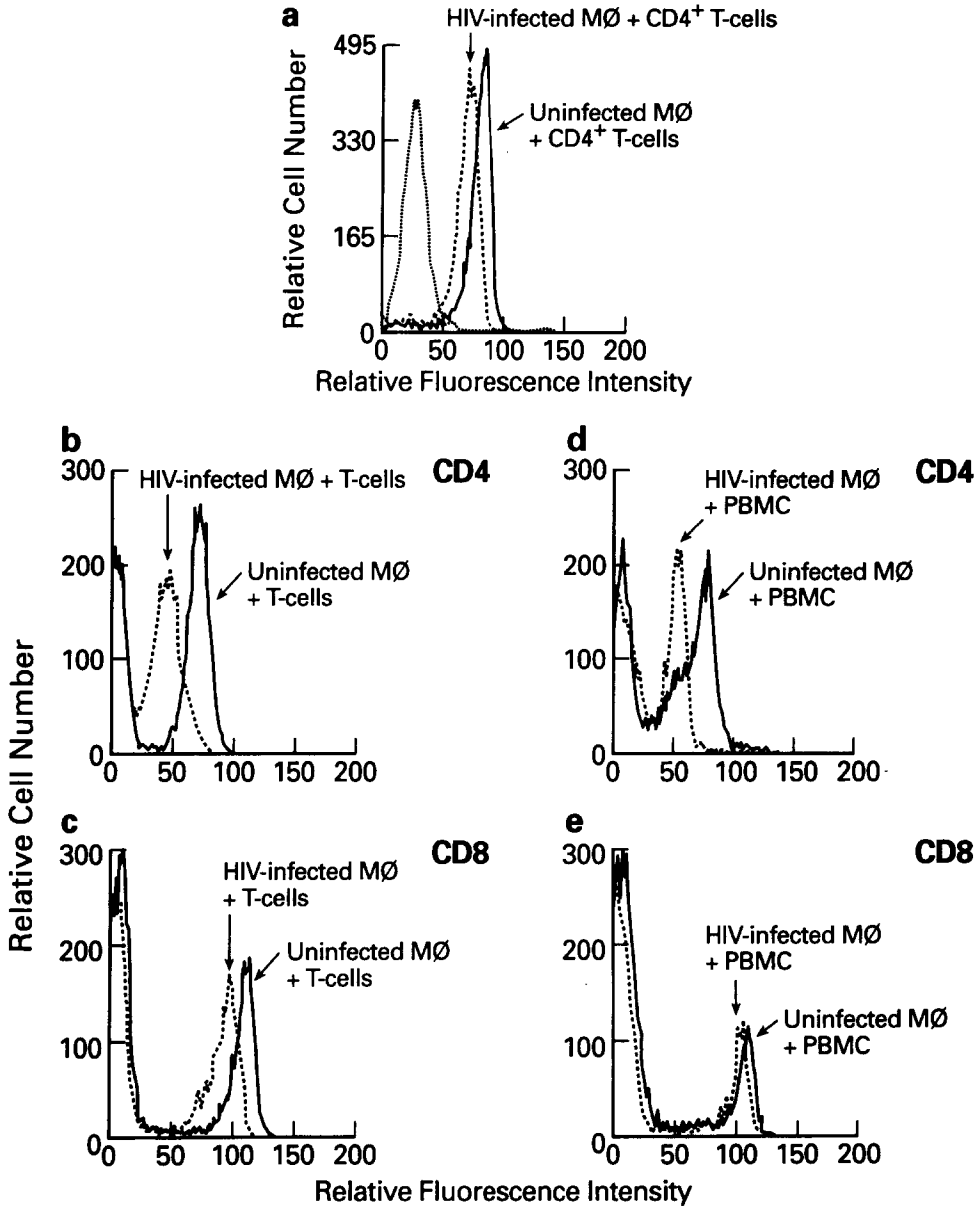

Fig. 3. CD4 and CD8 expression on T-cells co-cultured with uninfected or HIV-infected monocytes. CD4+ T-cells, total T-cells, or PBMC co-cultured with uninfected or HIV-infected monocytes for 3 days were recovered, washed 3 times with cold PBS, and incubated with CD3-FITC/CD4-PE or CD3-FITC/CD8-PE mAb at 4°C for 30 min. Cells were then washed 3 times with cold PBS, fixed with 4% paraformaldehyde, and analyzed by flow cytometry. (a) CD4 expression on purified CD4+ T-cells; (b) CD4 expression on gated CD8− cells in total T-cell population; (c) CD8 expression on gated CD8+ cells in total T-cell population; (d) CD4 expression on gated CD8− cells in PBMC; and (e) CD8 expression on gated CD8+ cells in PBMC. Solid lines in all histograms represent expression of CD4 or CD8 on T-cells co-cultured with uninfected monocytes, broken lines correspond to expression of CD4 or CD8 on T-cells co-cultured with HIV-infected monocytes, and dotted lines represent histograms derived from T-cells stained with isotype matched control IgG.

ciated factors on HIV-infected monocytes may down-modulate CD4 expression on T-cells, and alter the interaction between MHC class II molecules and TCR which is required for normal T-cell stimulation.

4. Discussion

Previous studies have demonstrated defects in antigen presentation by monocytes from HIV-infected individuals [7]. The mechanism by which HIV infection may alter the capacity of monocytes to stimulate T-cells, however, has remained undefined so far. To understand the mechanisms involved in the impaired accessory function by HIV-infected monocytes, we have investigated how HIV infection affects the ability of monocytes to stimulate and present antigen to CD4+ T-cells. Our results indicate that reduced T-cell stimulation by HIV-infected monocytes was mediated by factors associated with monocyte membranes, and not by soluble factors secreted by HIV-infected cells. In addition, the fact that paraformaldehyde-fixed monocytes as well as membranes from HIV-infected monocytes elicited a similar response further supports this notion. Therefore, it is likely that HIV-induced host factor(s) on monocytes might alter the interactions between MHC-class II molecules with TCR on CD4+ T-cells required for T-cell stimulation, and it is so indicated as 'host factor' in the proposed model depicted in Fig. 4. In this proposed mod-

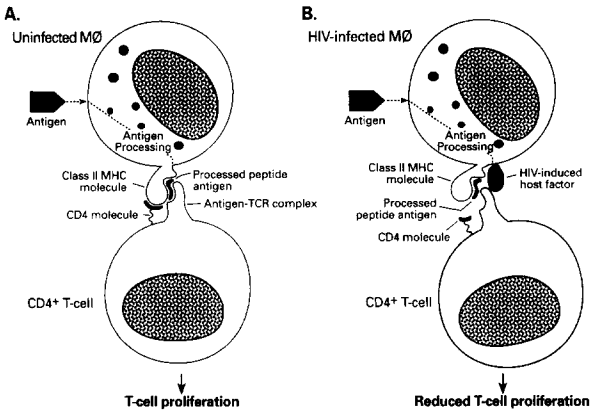

Fig. 4. Schematic representation of altered T-cell-monocyte interaction by HIV-induced host factor(s) on monocytes. (A) Monocyte-induced T-cell stimulation; (B) reduced T-cell stimulation by HIV-infected monocytes. Altered HLA-DR-TCR interaction between HIV-infected monocytes and CD4+ T-cells due to down-modulation of CD4 by HIV-induced host factor(s) on monocyte are shown to the right.

el. HIV-induced host factors may contribute to reduced T-cell proliferation by: (a) altered MHC-class II binding with TCR by creating steric hindrance between these molecules; or (b) inducing inappropriate signaling in T-cells to down-modulate CD4 molecule which supports HLA-DR-TCR interactions; or (c both. These processes could contribute to defective immune function of CD4+ T-cells in HIV infection. To our knowledge, this is the first report suggesting a possible involvement of HIV-induced host factors in viral immunopathogenesis.

Since monocytes from HIV-infected patients have demonstrated defective antigen presentation by monocytes with the progression of HIV disease [7,12], we examined the accessory function of HIV infected monocytes infected with HIV at various MOI, and at various times after HIV infection. We have demonstrated that reduced capacity of HIV-infected monocytes to stimulate and present antigens to CD4+ T-cells was directly related to viral load in the infected monocytes. With time, CD4+ T-cells in co-culture with HIV-infected monocytes were less responsive to monocyte-mediated stimulation. Thus, consistent with the clinical observations, this system provides a novel model to examine the mechanisms involved in the altered accessory function of HIV-infected monocytes.

T-cells co-cultured with HIV-infected monocytes revealed decreased CD4 expression as compared to those cultured with uninfected monocytes. Similar results were obtained when total T-cells or PBMC were incubated with HIV-infected monocytes with no significant altered CD8 expression. It is, therefore, likely that reduced T-cell proliferation may be due to an insufficient level of CD4 molecule required to support HLA-DR-TCR interactions as shown in Fig. 4. We next examined the likelihood of HLA-DR dysregulation on monocytes that could also alter their interactions with TCR on CD4+ T-cells, and reduce their ability to stimulate T-cells. Our data, however, indicate no significant difference in the level of HLA-DR between uninfected and HIV-infected monocytes (not shown). These findings are consistent with those reported earlier by other investigators [20]. Thus, the inability of HIV-infected monocytes to stimulate CD4+ T-cells may not be due to altered HLA-DR expression on infected monocytes.

To investigate whether reduced T-cell proliferation by HIV-infected monocytes was due to participation of gp120 expressed on the surface of HIV-infected monocytes [18], we examined the ability of anti-HIV-gp120 Ab to block this effect. Our results indicate that inclusion of anti-gp120 Ab in MLR had no effect on HIV-infected monocyte-induced reduced T-cell proliferation, indicating the possible involvement of viral gp120 to be less likely. The proposed role of membrane-associated molecules on HIV-infected monocytes for reduced T-cell proliferation was supported by experiments where the membranes isolated from infected monocytes or paraformaldehyde-fixed monocytes elicited immune responses in T-cells similar to those induced by monocytes productively infected with HIV. This concept was further supported by experiments where uninfected monocytes partially restored stimulation of T-cells that had been co-cultured with HIV-infected monocytes. These results indicate that defective T-cell response to HIV-infected monocytes may be a reversible phenomenon. Thus, based on these observations, it is reasonable to hypothesize that HIV-induced cellular factors may transduce inappropriate signaling in helper T-cells resulting in the defective immune response. However, additional experimental evidence is needed to support this hypothesis, and is currently underway in our laboratory. Furthermore, the effect of HIV-induced host factors on costimulation of T-cells via B-7/CD28 interaction which is essential for optimal T-cell stimulation [21–24], is being examined. Taken together, these studies may provide better insights into the mechanisms for dysregulation of immune system in HIV infection.

The data presented in this report suggest that HIV infection of monocytes reduced their capacity to stimulate CD4+ T-cells. This effect was due to the involvement of cellular factors associated with the membrane of HIV-infected monocytes, and was not the result of viral or soluble factors secreted by infected cells. In our proposed model, HIV-infected monocytes are postulated to express HIV-induced host factors on their surface which can perturb monocyte-CD4+ T-cell interactions by down-modulating CD4 expression resulting in reduced T-cell proliferation. Thus, continuous exposure of HIV-infected monocytes/macrophages with peripheral blood T-cells may result in altered regulation of immune function seen in patients with AIDS.

Acknowledgements: We would like to thank Drs. Pravina Mattoo, Priti Mehrotra and Ewa Matczak for critical reading of the manuscript.

References

[1] Meltzer, M.S., Skillman, D.R., Gomatos, P.J., Kalter, D.C. and Gendelman, H.E. (1990) Annu. Rev. Immunol. 8, 169–194.
[2] Bender, B.S., Davidson, B.L., Kline, R., Brown, C. and Quinn, T.C. (1988) Rev. Infect. Dis. 10, 1142–1154.
[3] Dhawan, S., Weeks, B., Soderland, C., Schnaper, H.W., Toro, L.A., Asthana, S., Hewlett, I.K., Stetler-Stevenson, W.G., Yamada, S., Yamada, K.M. and Meltzer, M.S. (1995) J. Immunol. 154, 422–432.
[4] Konig, S., Gendelman, H.E., Orenstein, J.M., Dal Conto, M.C., Pezeshkpour, G.H., Youngbluth, M., Janotta, F., Aksamit, A., Martin, M.A. and Fauci, A.S. (1986) Science 233, 1089–1093.
[5] Gartner, S., Markovitz, P., Markovitz, D.M., Betts, R.F. and Popovic, M. (1988) J. Am. Med. Assoc. 256, 2365–2371.

[6] Nelson, C.A., Harding, C.V. and Unanue, E.R. (1992) Cold Spring Harbor Symp. Quant. Biol. 57, 557–563.
[7] Baldwin, G.C., Fleischman, J., Chung, Y., Koyagani, Y., Chen, I.S.Y. and Golde, D.W. (1990) Proc. Natl. Acad. Sci. USA 87, 3933–3937.
[8] Rich, E.A., Toossi, Z., Fujiwara, H., Hanigosky, R., Lederman, M.M. and Ellner, J.J. (1988) J. Lab. Clin. Med. 112, 174–181.
[9] Prince, H.E., Moody, D.J., Shubin, B.I. and Lahey, J.L. (1985) J. Clin. Immunol. 5, 21–25.
[10] Ennen, J., Seipp, I., Norley, S.G. and Kurth, R. (1990) Eur. J. Immunol. 20, 2451–2456.
[11] Petit, A.J., Tersmette, M., Terpstra, F.G., De Goede, R.E., Van Lier, R.A. and Miedema, F. (1988) J. Immunol. 140, 1485–1489.
[12] Lane, H.C., Masur, H., Edgar, L.C., Whalen, G., Rook, A.H. and Fauci, A.S. (1983) N. Engl. J. Med. 309, 453–458.
[13] Meyaard, L., Schuitemaker, H. and Miedema, F. (1993) Immunol. Today 14, 161–164.
[14] Wahl, L., Katona, I.M., Wilder, R.L., Winter, C.C., Haraoui, B., Scher, I. and Wahl, S.M. (1984) Cell Immunol. 85, 373–383.
[15] Gendelman, H.E., Orenstein, J.M., Martin, M.A., Ferrua, C., Mitra, R., Phipps, T., Wahl, L.M., Lane, H.C., Fauci, A.S., Burke, D.S., Skillman, D. and Meltzer, M.S. (1988) J. Exp. Med. 167, 1428–1441.
[16] Lin, P.H., Selinfreund, R., Wakshull, E. and Wharton, W. (1987) Biochemistry 26, 731–736.
[17] Dhawan, S., Toro, L.A., Jones, B.E. and Meltzer, M.S. (1992) J. Leukocyte Biol. 52, 244–248.
[18] Busso, M., Thornthwaite, J. and Resnick, L. (1991) AIDS 5, 1425–1432.
[19] Janeway, C.A., Jr. (1991) Semin. Immunol. 3, 153–160.
[20] Melendez-Guerrero, L.M., Nicholson, J.K. and McDougal, J.S. (1990) AIDS Res. Hum. Retroviruses 6, 731–741.
[21] Weaver, C.T. and Unanue, E.R. (1990) Immunol. Today 11, 49–55.
[22] Ledbetter, J.A., Imboden, J.B., Schieven, G.L., Grosmaire, L.S., Rabinovitch, P.S., Lindsten, T., Thompson, C.B. and June, C.H. (1990) Blood 75, 1531–1539.
[23] Chen, C., Gault, A., Shen, L. and Nabavi, N. (1994) J. Immunol. 152, 4929–4936.
[24] Hathcock, K.S., Laszlo, G., Pucillo, C., Linsley, P.S. and Hodes, R.J. (1994) J. Exp. Med. 180, 631–640.