Human immunodeficiency virus (HIV) infection results in a significant quantitative loss of CD4+ T cells relatively late after seroconversion (9, 28). However, qualitative differences in the functional performance of peripheral blood mononuclear cells (PBMCs) from HIV-infected subjects can be detected much earlier. Loss or reduction of T-cell proliferative capacity to in vitro stimulation is one of these qualitative changes (4, 13, 20, 31). Decreased proliferation of T cells from HIV-infected individuals has been measured in response to in vitro stimulation with CD3 monoclonal antibody (MAb), pokeweed mitoxagen, and recall antigens (3, 33, 34). The response to phytohemagglutinin (PHA) remains unaffected in the early phases but is significantly reduced later in infection (8, 14). It has been reported that T-cell proliferation in response to stimulation with CD3 and CD3+CD28 MAbs decreases shortly after seroconversion, before the decline in CD4+ T-cell number is observed (10, 23, 33). It has also been demonstrated that loss of T-cell reactivity to CD3 and CD3+CD28 MAbs in vitro is a strong predictive marker for progression to AIDS, independent of decline in CD4 counts (32, 33). Thus, T-cell proliferative capacity is an important independent predictor of progression to HIV disease (31, 33) and also can serve to monitor immunological improvement after therapy (1, 26).

The most commonly used method to determine T-cell proliferative capacity is based on measuring tritiated thymidine ([3H]TdR) incorporation into the DNA of proliferating cells. This method requires radioactive facilities, it has drawbacks in places where these facilities and the management of waste are not available. In addition, proliferation as measured by [3H]TdR incorporation does not give information on which subsets of cells are in fact proliferating to the in vitro stimuli.

CD38 is a type II transmembrane glycoprotein originally identified by the MAb OKT 10 (30). It plays a role in lymphocyte adhesion, proliferation, and cytokine production (7). Most peripheral T and B cells, as well as red blood cells, are CD38 negative (18, 30). However, CD38 is expressed on activated lymphocytes; thus, it has been used as an activation marker of T cells. In HIV-infected individuals, the in vivo expression of CD38 on T cells is elevated and reported to be predictive of progression of HIV disease to AIDS and death (2, 12, 15, 16, 27). CD38 was also expressed on immunoglobulin (Ig)-secreting plasma B cells. Finally, CD38 has also been detected on immature cells, i.e., thymocytes and germinal center B cells (17).

In this paper, two alternative methods were evaluated for assessment of T-cell proliferative capacity in response to in vitro stimulation. These methods involved flow cytometric measurement of CD38 expression on T cells and enzyme-
linked immunosorbent assay (ELISA) determination of 5-bromo-2′-deoxyuridine (BrdU) incorporation into newly synthesized DNA of proliferating T cells. BrdU is a pyrimidine analogue and is incorporated in place of thymidine into DNA of proliferating cells. It is measured by ELISA using peroxidase-labeled anti-BrdU antibody.

The two methods were tested for applicability on both HIV-negative and HIV-positive samples and compared to the commonly used [3H]Tdr incorporation assay as a “gold standard.”

**MATERIALS AND METHODS**

**Study population.** Heparin venous blood samples were collected from HIV-1-infected participants \( n = 26 \); medians: CD4 count, 330/μl; CD8 count, 870/μl; CD4-to-CD8 ratio, 0.45) of the Amsterdam cohort study on HIV-1 infection and AIDS and from healthy Dutch blood donors \( n = 18 \); medians: CD4 count, 993/μl; CD8 count, 506/μl; CD4-to-CD8 ratio, 2.0) after informed consent. 993/n 5 CD4-to-CD8 ratio, 0.45) of the Amsterdam cohort study on HIV-1 infected participants (53x346). Anti-CD3 six 96-well plate wells were pooled in order to have sufficient numbers of cells for

**Measurement of CD38-positive T cells.** The two methods were tested for applicability on both HIV-negative and HIV-positive samples, and compared to the commonly used [3H]Tdr incorporation assay as a “gold standard.”

**RESULTS**

**Determination of optimal in vitro stimulus.** To determine the optimal conditions for using CD38 as a readout for T-cell proliferation, PBMCs were cultured for 3 days, a stimulation period which was established to be optimal for the [3H]Tdr incorporation assay (5). Three different stimuli were used: (i) CD3 MAb, (ii) CD3+CD28 MAb, and (iii) PHA. Table 1 shows the correlation of absolute numbers of CD38 + T cells with counts per minute determined by [3H]Tdr incorporation assays in response to the various stimuli. Stimulation by the combination of CD3+CD28 MAb resulted in the strongest \( r = 0.75 \) overall association (HIV-negative samples, \( r = 0.65 \); HIV-positive samples, \( r = 0.71 \)). Stimulation with CD3 MAb yielded correlations of 0.84 (for HIV−) and 0.15 (for HIV+), with an overall correlation of 0.71, and stimulation with PHA yielded correlations of 0.55 (for HIV−) and 0.24 (for HIV+), with an overall correlation of 0.46. Thus, the combination of CD3+CD28 MAb was used in all of the following experiments, which measured CD38 expression. Figure 1 details the correlation pattern of percentage (Fig. 1A) and absolute numbers (Fig. 1B) of CD3+CD28 + T cells with [3H]Tdr incorporation in HIV-positive (\( n = 26 \)) versus HIV-negative (\( n = 18 \)) individuals. As shown in Fig. 1A, the use of the percentage of CD3+ cells expressing CD38 as a readout could discriminate well between the proliferation capacity of HIV-negative and HIV-positive subjects and has an overall good correlation with the [3H]Tdr incorporation assay (\( r = 0.81 \)). However, as a result of a high percentage of CD3+ cells expressing CD38 in response to the in vitro stimulation in most of the HIV−
subjects, the correlation with the \[^{3}H\]TdR incorporation assay for this group looks poor (\(r = 0.03\) for HIV\(^{-}\) versus \(r = 0.87\) for HIV\(^{+}\)). Therefore, the absolute number of CD3\(^{+}\) CD38\(^{+}\) cells is preferred to be used as a readout for comparison with the \[^{3}H\]TdR incorporation assay.

**Determination of optimal stimulation time.** Time-response experiments were performed to determine the optimal period of stimulation using the above combination of CD3\(^{+}\) CD28 MAb. As shown in Table 2, the best correlation of absolute numbers of CD3\(^{+}\) T cells expressing CD38 with \[^{3}H\]TdR incorporation, including a proper signal-to-noise ratio, was found after 3 days of stimulation with CD3\(^{+}\) CD28 (\(r = 0.96\) for HIV\(^{+}\); \(r = 0.84\) for HIV\(^{-}\)).

**Analysis of CD38 expression on T cells after in vitro stimulation.** Figure 2 shows typical fluorescence-activated cell sorter (FACS) analysis dot plots of CD38 expression on CD3\(^{+}\) T cells for an HIV-positive individual (Fig. 2B) and an HIV-negative individual (Fig. 2C) after 3 days of culture with CD3\(^{+}\) CD28 MAb. A dot plot from an isotype control tube is also shown in Fig. 2A. Invariably, the PBMCs of HIV-positive individuals show lower proportions and absolute numbers of T cells expressing CD38 after stimulation compared to those of HIV-negative individuals (in this case, 60.6\% versus 97.2\%).

**Kinetics of CD3\(^{+}\) and CD3\(^{+}\) CD38\(^{+}\) T cells.** We followed the kinetics of the percentage and number of CD3\(^{+}\) T cells and their CD38-expressing subsets over 4 days in anti-CD3\(^{+}\) CD28-stimulated cells from 12 HIV-positive and -negative subjects. As shown in Fig. 3, the percentage and number of CD3\(^{+}\) T cells increase continuously. The percentage of CD3\(^{+}\) CD38\(^{+}\) T cells also increases in parallel, and the increase seems to be sharp after 2 days of culture.

**BrdU ELISA.** The BrdU ELISA procedure, which utilizes the incorporation of BrdU into the DNA of proliferating cells, was performed on Ficoll-Isopaque-isolated PBMCs only. The method was not found to be suitable for whole-blood culture, because of the high background due to red blood cells interfering with the ELISA reader OD\(_{450}\) measurements. As shown in Fig. 4, the OD\(_{450}\) results of the BrdU ELISA correlated strongly (overall, \(r = 0.82\); HIV\(^{-}\), \(r = 0.83\); HIV\(^{+}\), \(r = 0.96\)) with the counts per minute of the \[^{3}H\]TdR incorporation assay. This correlation was further improved (overall, \(r = 0.92\))

![FIG. 1. Correlation of percentage (A) and number (B) of CD3\(^{+}\) cells expressing CD38 with a standard 3-day \[^{3}H\]TdR incorporation assay for 18 HIV\(^{-}\) (●) and 26 HIV\(^{+}\) (○) PBMCs stimulated with CD3\(^{+}\) CD28 MAb.](image)

### Table 2. Comparison of median percentages and absolute numbers of CD3\(^{+}\) T cells expressing CD38, after different periods of stimulation, with median \[^{3}H\]TdR incorporation after 3 days of stimulation of whole-blood samples with CD3\(^{+}\) CD28 MAb

| Stimulation period | Subject group (n) | % CD38\(^{+}\) T cells | Absolute no. of CD38\(^{+}\) T cells (10\(^{4}\)) | cpm (10\(^{4}\)) | Correlation (\(r^b\)) | \(P\) |
|--------------------|-------------------|--------------------------|---------------------------------------------|-----------------|------------------------|------|
| 1 day              | HIV\(^{-}\) (4)    | 21 (15–27)               | 8.8 (5.4–14.9)                             | -0.66           | NS\(^c\)               |      |
|                    | HIV\(^{+}\) (8)    | 11 (6–20)                | 3.5 (1.2–9.2)                              | -0.02           | NS\(^c\)               |      |
|                    | All (12)           | 16 (6–27)                | 5.7 (1.2–14.9)                             | 0.18            | NS                     |      |
| 2 days             | HIV\(^{-}\) (4)    | 37 (34–59)               | 17.1 (15.1–19.3)                           | -0.71           | NS                     |      |
|                    | HIV\(^{+}\) (8)    | 23 (17–61)               | 6.7 (4.5–36.1)                             | 0.71            | 0.04                   |      |
|                    | All (12)           | 31 (17–61)               | 12.3 (4.5–36.1)                            | 0.69            | 0.01                   |      |
| 3 days             | HIV\(^{-}\) (4)    | 81 (74–92)               | 80.2 (51.0–80.8)                           | 3.2 (1.8–3.9)   | 0.84                   | <0.001|
|                    | HIV\(^{+}\) (8)    | 75 (24–96)               | 44.2 (5.6–135.4)                           | 1.3 (0.06–4.5)  | 0.96                   | <0.001|
|                    | All (12)           | 81 (24–96)               | 51.7 (5.6–135.4)                           | 1.6 (0.06–4.5)  | 0.91                   | <0.001|
| 4 days             | HIV\(^{-}\) (4)    | 96 (84–97)               | 174.9 (90.2–238.2)                         | 0.09            | NS                     |      |
|                    | HIV\(^{+}\) (8)    | 93 (47–98)               | 110.6 (13.6–399.8)                         | 0.98            | <0.001                 |      |
|                    | All (12)           | 94 (47–98)               | 124.7 (13.6–399.8)                         | 0.83            | <0.001                 |      |

\(^{a}\) 95% ranges are shown in parentheses.  
\(^{b}\) Correlations are between counts per minute and absolute numbers of CD38\(^{+}\) T cells.  
\(^{c}\) NS, not significant.
when the final substrate reaction supernatant was transferred into a new 96-well plate before measuring OD_{450}.

**DISCUSSION**

The [³H]TdR incorporation assay is widely applied as a measure of T-cell proliferation in vitro and thus as an indirect quantification of T-cell activation. However, this technology gives only information on the overall proliferative responses, not detailing the specific cell subsets involved in these responses. In addition, this technology is only applicable in laboratories where radioactive facilitates are present and proper waste management is implemented.

In this paper, two alternative methods to measure T-cell stimulation are described, one which measures the expression of the T-cell activation marker CD38 and the other which measures BrdU incorporation into newly generated DNA.

The optimal reaction conditions for using CD38 expression as an alternative readout to [³H]TdR incorporation proved to be 3 days of stimulation with the combination of CD3+CD28 MAbs. When CD3 MAb alone was used, the overall stimulation and also its correlation with [³H]TdR incorporation was lower. This is in accordance with previous reports that, for optimal mitogenic stimulation, signaling not only through the T-cell receptor (via CD3) but also through costimulatory molecules (like CD28) is essential (21).

As presented in this report, CD38 expression paralleled DNA synthesis as measured by the [³H]TdR incorporation assay. This observation may indicate that the cells expressing CD38 after the in vitro stimulation are proliferating. Paradoxically, T cells obtained from HIV+ patients with high expression of CD38 in vivo were found to be confined to the non-proliferative phase of the cell cycle (22). This may suggest that T cells expressing CD38 have different characteristics in terms of proliferation depending on whether the activation is in vivo or in vitro.

Studies have reported that CD38 binding elicits activation and proliferation programs in T cells, and the involvement of CD38 in signal transduction and cell adhesion is indicated (11). Furthermore, an important association between a reduced T-cell proliferation and reduced percentage of CD38 T cells has been shown for people with depression (6). Our results may further support the importance of CD38 in T-cell proliferation.

We have shown that measurement of CD38 expression can

![Fig. 2. Representative dot plots of PBMCs stained with aCD3-PE and aCD38-FITC. Data were obtained with samples from an isotype control tube (A), an HIV-positive subject (B), and an HIV-negative subject (C) after 3 days of stimulation with CD3+CD28 MAbs.](image)

![Fig. 3. Kinetics of percentage and absolute number of CD3+ and CD3+CD38 T cells over 4 days in cells stimulated with anti-CD3+CD38. Each point represents median percentage and absolute numbers from 12 HIV-negative and HIV-positive subjects.](image)
be exploited as an alternative readout for the capacity of T cells to proliferate in response to different stimuli. The correlation of the absolute number of CD38+ T cells with the [3H]TdR assay varied for the three stimuli used, being higher for the CD3+CD28 MAbs. Samples from a group of subjects different from those presented in Fig. 1 were used for this assay.

We observed that the BrdU ELISA also showed a good association with the [3H]TdR incorporation (overall correlation, \( r = 0.82 \)). The correlation was further improved (\( r = 0.92 \)) when the final reaction supernatant was transferred to a new 96-well plate before measuring OD_{450}. This may be due to background signals caused by the fixed cells.

The CD38 method needs equal investments in terms of laboratory equipment as compared to the [3H]TdR incorporation assay. However, no radioactivity is used, and in addition, the nature of the proliferating T-cell subsets can be studied in much more detail by combining CD38 MAbs with other reagents for FACS analysis. Furthermore, the signal-to-noise ratio is higher (up to 20:1) than that of the BrdU ELISA (up to 10:1). The broad expression of CD38 on stimulated T cells may indicate its potential use for detection of low-frequency responses.

The BrdU method is clearly much more economical than the above two methods; however, its sensitivity is limited by the OD_{450} readout range (between 0.000 and 3.000) and it does not allow for subspecifying the contribution of certain T-cell subsets to the overall proliferation signal. Also, the assay proved to be only feasible on isolated PBMCs and it is not suitable for whole-blood cultures, because of the high background due to the presence of red blood cells interfering with the OD_{450} reading. However, it is worth mentioning that the BrdU method is currently being used successfully in our laboratory to measure proliferation responses in PHA- and purified protein derivative-stimulated PBMCs from both HIV-negative as well as HIV-positive Ethiopians (M. Legesse, personal communication).

In conclusion, the data presented in this paper show that the BrdU method, followed by the CD38 expression method, showed a significant agreement with the widely used [3H]TdR incorporation assay in measuring T-cell proliferation in response to stimulation by PHA, anti-CD3, and anti-CD3+CD28, indicating that both methods can be used as alternative techniques to measure in vitro T-cell proliferative capacity in response to the above stimulants. However, the use of these methods for measuring low-frequency responses (responses to recombinant antigens) needs to be assessed.

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REFERENCES

1. Autran, B., G. Carcelain, T. S. Li, C. Blanc, D. Mathiez, R. Tubiana, C. Katlama, P. Debre, and J. Leibowitch. 1997. Positive effects on combined antiretroviral therapy on CD4+ T cell homeostasis and function in advanced HIV disease. Science 277:112–116.

2. Autran, B., and J. V. Gisbert. 1992. Activated CD8+ cells in HIV-related diseases, p. 171–184. In G. Janossy, B. Autran, and F. Miedema (ed.), Immunodeficiency in HIV infection and AIDS. Karger Basel, Basel, Switzerland.

3. Ballet, J., L., J., Coudrec, C. Ribian-Herzog, C. Duval-Roy, F. Janier, M. Danon, P. P. Clauvel, and M. Seligmann. 1988. Impaired T-lymphocyte dependent immune responses to microbial antigens in patients with HIV-1 associated persistent generalized lymphadenopathy. AIDS 2:91–297.

4. Benton, J., C. Tsoukas, J. A. McCutchan, S. E. Spector, D. B. Richman, and J. H. Vaughan. 1989. Impairment in T-lymphocyte responses during early infection with the human immunodeficiency virus. J. Clin. Immunol. 9:159–168.

5. Blomma, E., M. T. L. Ross, J. L. A. M. van Heijst, J. M. J. L. J. Vossen, and P. T. A. Schellekens. 1989. Whole-blood lymphocyte cultures. J. Immunol. Methods 122:161–167.
6. Castle, S., S. Wilkins, E. Heck, K. Tanzy, and J. Fahey. 1995. Depression in caregivers of demented patients is associated with altered immunity: impaired proliferative capacity, increased CD8+, and a decline in lymphocytes with surface signal transduction molecules (CD8+), and a cytotoxicity marker (CD56+CD8+). Clin. Exp. Immunol. 101:487–493.

7. Cesano, A., S. Visonneau, S. Deaglio, F. Malavasi, and D. Santoli. 1998. Role of CD38 and its ligand in the regulation of MHC-nonrestricted cytotoxic T-cells. J. Immunol. 160:1106–1115.

8. Clerici, M., N. I. Stocks, R. A. Zajac, R. N. Boswell, D. R. Lucey, C. S. Via, and G. M. Shearer. 1989. Detection of three distinct patterns of T helper cell dysfunction in asymptomatic human immunodeficiency virus-seropositive patients. J. Clin. Invest. 84:1892–1899.

9. De Wolf, F., J. M. A. Lange, J. T. M. Houweuling, R. A. Coutinho, P. T. Schellekens, J. van der Noorda, and J. Goudsmit. 1988. Numbers of CD4+ cells and the levels of core antigens of and antibodies to the human immunodeficiency virus as predictors of AIDS among seropositive homosexual men. J. Infect. Dis. 158:615–622.

10. Dolan, J. M., M. Clerici, S. P. Blatt, C. W. Hendrix, G. P. Melcher, R. N. Bowtell, T. M. Freeman, W. Ward, R. Hensley, and G. M. Shearer. 1995. In vitro T-cell culture, delayed-type hypersensitivity skin testing and CD4 T-cell subset phenotyping independently predict survival time in patients infected with human immunodeficiency virus. J. Infect. Dis. 172:79–87.

11. Funaro, A., C. G. Spagnoli, C. M. Ansieou, M. Alessio, S. Ruggero, D. Delia, M. Zaccolo, and F. Malavasi. 1990. Involvement of the multinegative CD38 molecule in a unique pathway of cell activation and proliferation. J. Immunol. 145:2390–2396.

12. Giorgi, J. V., H. N. Ho, K. Hirji, C. C. Chou, L. E. Hultin, S. O'Rourke, L. Park, J. B. Margolich, J. Ferbas, J. P. Phair, and the Multicenter AIDS Cohort Study Group. 1994. CD8+ lymphocyte activation at human immunodeficiency virus type 1 seroconversion: development of HLA-DR+CD38+CD8+ cells is associated with subsequent stable CD4+ cell levels. J. Infect. Dis. 170:775–781.

13. Gruters, R. A., F. G. Terpstra, R. De Jong, C. J. M. Van Noesel, R. A. W. Van Noesel, M. Zaccolo, and F. Malavasi. 1990. Kinetics of activation antigen expression by in vitro stimulated human T cells and the levels of core antigens of and antibodies to the human immunodeficiency virus type 1 seroconversion: development of HLA-DR+CD38+CD8+ cells is associated with subsequent stable CD4+ cell levels. J. Immunol. 142:1874–1880.

14. Holter, W., O. Majdik, K. Liszka, H. Stockinger, and W. Knapp. 1985. Kinetics of activation antigen expression by in vitro stimulated human T lymphocytes. Cell. Immunol. 90:322–330.

15. Hong, H. N., L. E. Hultin, R. T. Mitsuyasu, J. L. Matud, M. A. Hausner, D. Bockstoce, C. C. Chou, S. O'Rourke, J. M. G. Taylor, and J. V. Giorgi. 1993. Circulating HIV-specific CD8+ cytotoxic T cells express CD38 and HLA-DR antigens. J. Immunol. 150:3070–3079.

16. Jackson, G. D., and I. J. Bell. 1985. Qualitative analysis of immune function in HIV infection: analysis of in vivo activated lymphocytes. Clin. Exp. Immunol. 102:481–486.

17. McCloskey, T. W., T. Cavalieri, S. Bakshi, R. Harper, J. Fagin, N. Kohn, and S. Pahwa. 1997. Immunophenotyping of T lymphocytes by three-color flow cytometry in healthy newborns, children and adults. Clin. Immunol. Immunopathol. 84:46–55.

18. Messequ, T., M. Abdulkadir, A. L. Fontanet, B. Petros, D. Hamann, M. Koot, M. T. L. Roos, P. T. A. Schellekens, F. Miedema, and T. F. Rinke de Wit. 1999. Reduced naive and increased activated CD4 and CD8 cells in healthy adult Ethiopians compared with their Dutch counterparts. Clin. Exp. Immunol. 115:443–450.

19. Miedema, F., A. J. C. Petit, F. G. Terpstra, J. K. E. Schattenkerk, F. De Wolf, B. J. M. Al, M. Roos, J. M. A. Lange, S. A. Danner, J. Goudsmit, and P. T. A. Schellekens. 1998. Immunological abnormalities in human immunodeficiency virus (HIV)-infected asymptomatic homosexual men. HIV affects the immune system before CD4+ T helper cell depletion occurs. J. Clin. Invest. 82:1908–1914.

20. Pakker, N. G., M. T. L. Roos, R. van Leeuwen, M. D. de Jong, M. Koot, P. Reiss, J. M. A. Lange, F. Miedema, S. A. Danner, and P. T. A. Schellekens. 1997. Patterns of T-cell repopulation, virus load reduction, and restoration of T-cell function in HIV-infected persons during therapy with different antiretroviral agents. J. Acquir. Immune Defic. Syndr. 16:318–326.

21. Plagker, S., H. Z. Bass, P. Nishanhan, J. Thomas, N. Aziz, R. Detels, J. King, W. Cumberland, M. Kemeny, and J. Fahey. 1999. The prognostic significance in HIV infection of immune activation represented by cell surface antigen and plasma activation marker changes. Clin. Immunol. 90:238–246.

22. Polk, B. F., R. Fox, R. Brookmeyer, S. Kanchanaraksa, R. Kaslow, R. Visscher, C. Rinaldo, and J. Phair. 1987. Predictors of the acquired immunodeficiency syndrome developing in a cohort of seropositive homosexual men. N. Engl. J. Med. 316:61–66.

23. Prince, H. E., and E. R. Jensen. 1991. HIV-related alterations in CD8 cell subsets defined by in vitro survival characteristics. Cell. Immunol. 134:276–286.

24. Reinhart, E. L., P. C. Kung, G. Goldstein, R. H. Levey, and S. F. Schlossman. 1980. Discrimine stages of human intrathymic differentiation: analysis of normal thymocytes and leukemic lymphoblasts of T-cell lineage. Proc. Natl. Acad. Sci. USA 77:1588–1592.

25. Roos, M. T. L., F. Miedema, M. Koot, M. T. L. Roos, R. Van Leeuwen, M. D. De Jong, M. Koot, P. Reiss, J. M. A. Lange, F. Miedema, S. A. Danner, and P. T. A. Schellekens. 1995. T-cell function in vitro is an independent progression marker for AIDS in human immunodeficiency virus (HIV)-infected asymptomatic individuals. J. Infect. Dis. 171:531–536.

26. Roos, M. T. L., F. Miedema, M. A. P. Meinesz, N. A. M. De Leeuw, N. G. Pakker, J. M. A. Lange, R. A. Coutinho, and P. T. A. Schellekens. 1996. Low T-cell reactivity to combined CD3 plus CD28 stimulation is predictive for progression to AIDS correlation with decreased CD28 expression. Clin. Exp. Immunol. 105:409–415.

27. Schellekens, P. T. A., M. T. L. Roos, F. De Wolf, J. M. A. Lange, and F. Miedema. 1990. Low T-cell responsiveness to activation via CD3/TCR is a prognostic marker for AIDS in HIV-1 infected men. J. Clin. Immunol. 10:121–127.

28. Shearer, G. M., D. C. Bernstein, K. S. K. Tung, C. S. Via, R. Redfield, S. Z. Salabuddin, and R. C. Gallo. 1986. A model for the selective loss of major histocompatibility complex self restricted T-cell immune responses during the development of acquired immune deficiency syndrome (AIDS). J. Immunol. 137:2514–2521.

29. Van Lier, R. A. W., J. H. A. Boot, A. J. Verhoeven, E. R. De Groot, M. A. Brouwer, and L. A. Aarden. 1987. Functional studies with anti-CD3 heavy chain isolates with variant monoclonal antibodies. J. Immunol. 139:2873–2879.

30. Van Lier, R. A. W., M. Brouwer, and L. A. Aarden. 1988. Signals involved in T-cell activation. T-cell proliferation induced through the synergistic action of anti-CD28 and anti-CD2 monoclonal antibodies. Eur. J. Immunol. 18:167–172.