Immune functions in untreated and treated multiple sclerosis patients
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Chapter 2

Functional defects in peripheral blood T cells of multiple sclerosis patients.
Diminished in vitro responsiveness in accessory cell dependent activation systems

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Capítulo

A propósito de la problemática de la vivienda en el Perú.

Enrique Sánchez

Director del Ministerio de Vivienda

Resumen

La vivienda es un derecho fundamental y básico que debe ser satisfactorio, seguro y económico para todos los habitantes. Sin embargo, en el Perú, el acceso a la vivienda es un tema crítico y urgente.

La problemática de la vivienda en el Perú

La vivienda en el Perú es un tema de debate y discusión constante. La situación actual es que la demanda de viviendas es superior a la oferta disponible.

La situación de la vivienda en el Perú

La situación de la vivienda en el Perú es compleja y multifacética. Hay varias causas que contribuyen a esta situación, como la falta de inversión en vivienda, la inseguridad en la propiedad y la falta de políticas públicas efectivas.

La problemática de la vivienda en el Perú

La problemática de la vivienda en el Perú es una realidad que afecta a millones de personas. La vivienda es un derecho fundamental que debe ser garantizado para todos los habitantes.

La situación de la vivienda en el Perú

La situación de la vivienda en el Perú es un tema de preocupación y de acción urgente. La sociedad y las autoridades deben trabajar juntas para solucionar esta problemática.

Referencias

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PBMC could be seen in both groups, in comparison to the use of CD2 antibodies alone (Fig. 2D). Again, these responses did not differ between MS patients and healthy controls (median cpm controls 49,418, range 7393–80992; MS 32,847, range 1873–73,694).

3.3. No difference in accessory cell function between MS patients and healthy controls

Peripheral blood accessory cell function, as determined by the addition of irradiated PBMC to healthy donor PBL stimulated with soluble CD3 mAb, showed no difference between MS patients (n = 4) and healthy controls (n = 5) (Fig. 3). Furthermore, there was no difference in the percentage of CD14+ cells in PBMC between patients and controls (MS 12.0 ± 2.6; controls 9.6 ± 2.7).

3.4. No evidence for gross phenotypical changes in circulating T cell subsets in MS patients

Fig. 4 shows the distribution of the analyzed markers among the CD3+, CD4+ and CD8+ lymphocyte subsets in peripheral blood. No differences were found in expression of CD45RA, CD45R0, CD27 and CD26 between healthy controls and MS patients in the CD3+ and the CD8+ subset.

However, within the CD4+ subset, MS patients showed a significantly higher percentage of CD45RA+ cells (50.1% ± 18.8%) in comparison with healthy controls (38.6% ± 11.1%, P = 0.009). Although the percentage of CD4+CD45R0+ was somewhat lower in MS patients compared to healthy controls, this difference proved not to be significant. No differences between MS patients and healthy controls were found in the expression of the other markers within this subset.

4. Discussion

Defects in in vitro proliferative responses of peripheral blood T cells after stimulation with CD3 and CD2 mAb have been reported in a variety of systemic im-
Functional defects in peripheral blood T cells of multiple sclerosis patients

Diminished in vitro responsiveness in accessory cell dependent activation systems

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Abstract

Function and phenotype of peripheral blood (PB) T cells in multiple sclerosis (MS) patients were analyzed. In whole blood cultures, T cell proliferation of multiple sclerosis (MS) patients, using soluble CD3 mAb and CD2 mAb as stimulants, was reduced in comparison to healthy controls. A similar difference was seen when isolated PBMC were tested after stimulation with soluble CD3 mAb. However, in accessory cell-independent activation systems, i.e. after stimulation of PBMC with immobilized CD3 mAb or after co-stimulation with CD28 mAb, both patients and controls responded equally well. Phenotypical analysis of the circulating T cell population showed that there were no differences in the percentage of CD26+ (CD45R0+) or "effector" (CD4+CD45RO+CD27+) cells between MS patients and healthy controls. Finally, although MS patients did show an enhanced proportion of "naive" (CD4+CD45RA+) T cells, this did not correlate with the observed functional defects.

Key words: Multiple sclerosis; CD45RA; CD45R0; CD27; Whole blood culture

1. Introduction

Multiple sclerosis (MS) is the major human demyelinating disease. There is evidence, albeit circumstantial, which suggests that MS is a T cell-mediated disease of autoimmune origin (McFarland and Dhib Jalbut, 1989; Hintzen et al., 1992; Martin et al., 1992). In phenotypical studies, the presence of activated T cells has been clearly demonstrated when cells of the central nervous system were analyzed (Bellamy et al., 1985; Noronha et al., 1985; Hafler et al., 1985a; Oger et al., 1988; Sobel et al., 1988; Chofflon et al., 1989; Hintzen et al., 1991). However, contradictory results have been obtained from phenotypical analyses of peripheral blood T cells. Some authors have reported increases of CD4+CD45RA+ or CD26+ PB T cells (Rose et al., 1985; Morimoto et al., 1987; Porrini et al., 1992), but in other studies these findings could not be confirmed (Salonen et al., 1989; Ilonen et al., 1990).

In spite of these discrepant findings from phenotypical analyses, immunological dysfunctions of peripheral blood T cells from MS patients have consistently been reported. These functional defects could be demonstrated in autologous mixed lymphocyte reactions (auto-AMLR) (Hafler et al., 1985b; Hirsch, 1986; Baxevanis et al., 1987), in suppressor cell assays (Neighbour and Bloom, 1979; Antel et al., 1986; Oger et al., 1988), and in CD2 mAb-induced T cell activation systems (Reder et al., 1991). In the present study, we examined the in vitro proliferative response of PB T cells of MS patients in accessory cell-dependent systems, after stimulation with soluble CD3 mAb (van Lier et al., 1987). Functional assays were performed in whole blood cultures, in order to measure T cell responsiveness per standard volume of blood, and in standard cultures employing isolated PBMC, in order to measure the response per standard amount of PBMC. Because of
its simplicity and reproducibility the former assay is highly suitable for routine practice, and might be of clinical relevance (Bloemena et al., 1989). Responses in standard cultures were also measured in accessory cell-independent systems, by using either immobilized CD3 mAb (van Lier et al., 1989) or CD28 mAb in combination with soluble CD3 mAb or CD2 mAb (van Lier et al., 1988). Furthermore, we examined whether there is evidence for an altered activation and differentiation state of the circulating T cell population. Therefore, we analyzed the expression of the markers CD45RA, CD45R0, CD26 and CD27 within CD3+, CD4+ and CD8+ subsets.

2. Materials and methods

2.1. Subjects

Forty MS outpatients (7 male, 33 female) were recruited from the Department of Neurology of the Free University Hospital in Amsterdam. Mean age of MS patients was 45.6 years (range 27.0–67.0). Fifteen of these patients were in a relapsing–remitting phase of the disease, six in a secondary progressive phase, and 18 patients in a relapsing progressive phase. One patient had a primary progressive disease course. Mean Expanded Disability Status Scale (EDSS) was 5.2 (range 1.5–8.5).

Twenty-three individuals (11 male, 12 female), recruited from the outpatient department and the Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, served as healthy controls. Mean age of controls was 40.4 years (range 21.0–70.0). None of the patients or controls had been receiving immunomodulating agents for at least 1 year before the time of sampling. Subjects analyzed in smaller groups were included in all studies with bigger groups.

2.2. Blood samples and cell separation

Venous blood was collected in evacuated blood collection tubes containing sodium heparin. The samples were kept at room temperature and used within 24 h.

Peripheral blood mononuclear cells (PBMC) were isolated from heparinized blood by Ficoll-Isopaque density gradient centrifugation. Viability of the cells was over 95%, as indicated by Trypan blue exclusion.

2.3. Monoclonal antibodies

The following antibodies were used: CD3 mAb CLB-T3/4.E (IgE), CD3 mAb CLB-T3/3 (IgG2a), CD2 mAb CLB-T11.1/1, CLB-T11.1/2 and Hik27 (IgG1), CD28 mAb CLB-CD28/1 (IgG1), and CD14 mAb CLB-CD14. Double-colour immunofluorescence studies were performed with directly phycoerythin (PE)-conjugated CD3 (Leu-4), PE-labelled CD4 (Leu-3a) and PE-labelled CD8 (Leu-2a). These antibodies were used in combination with fluorescein isothiocyanate (FITC)-conjugated CD45RA mAb (2H4), FITC-labelled CD27 mAb (CLB-CD27/2), FITC-labelled CD45R0 mAb (UCHL-1), and FITC-labelled CD26 mAb (Ta1).

All mAb were raised and produced at the Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, except for PE-labelled mAbs which were purchased from Becton Dickinson Immunocytometry Systems (San Jose, CA), 2H4-FITC and Ta1-FITC which were obtained from Coulter (Coulter Electronics, Luton, UK), and UCHL-1-FITC which was obtained from Dako (Dakopatts, Glostrup, Denmark).

2.4. Whole blood culture

Whole blood cultures were performed as described (Bloemena et al., 1989), with the following modifications. Heparinized blood (10 μl) was diluted 1:15 (v/v) with Iscove's modified Dulbecco's medium (IMDM) supplemented with penicillin (100 U/ml), streptomycin (100 μg/ml) and β-ME (5 × 10−5 M). 150 μl of diluted blood was added to flat-bottom microtiter plates and stimulated with CD3 mAb CLB-T3/4.E (final dilution ascites 1:1000), or a triplet of CD2 mAb CLB-T11.1/1, CLB-T11.2/1 and Hik27 (final dilution ascites 1:1000), in the absence or presence of CD28 mAb CLB-CD28/1 (final dilution ascites 1:400).

Cells were stimulated for 4 days; 0.4 μCi/well of [3H]thymidine (200 mCi/mmol, Amersham, Buckinghamshire, UK) was added during the last 24 h of culture and the median cpm [3H]thymidine of triplicate cultures was measured.

2.5. Standard culture technique

Flat-bottom microtiter plates (Nunc, Roskilde, Denmark) were coated overnight at 4°C with CD3 mAb (CLB-T3/3, 5 μg/ml) in phosphate-buffered saline (PBS). The plates were washed twice with PBS before use. PBMC (5 × 10⁴) were cultured in a final volume of 200 μl IMDM, supplemented with 5% human pooled serum (HPS), penicillin, streptomycin and β-mercaptoethanol. Alternatively, PBMC were stimulated with CD3 mAb (CLB-T3/4.E final dilution ascites 1:1000), or a triplet of CD2 mAb (CLB-T11.1/1, CLB-T11.2/1 and Hik27 (final dilution ascites 1:1000), in the absence or presence of CD28 mAb (CLB-CD28/1, final dilution ascites 1:400). Cells cultured without stimuli served as negative controls; cell viability was > 95% in all experiments.
PBMC were stimulated for 4 days. \[^{3}H\]thymidine was added, and cpm were measured as described above.

2.6. Analysis of accessory cell function

Effects of accessory cells on T-cell responses were tested as described before (Miedema et al., 1988). Briefly, T cells were depleted for monocytes by counterflow centrifugation elutriation (Figdor et al., 1982) and additional adherence to plastic (2 h, 37°C). This population contained <1% CD14\(^+\) cells, as determined by flow cytometry. Thereafter, 1.6 \(\times 10^5\) 2000 rad irradiated PBMC of MS patients \((n = 4)\) and healthy controls \((n = 5)\) were added to 4 \(\times 10^4\) healthy donor T cells and stimulated with CD3 mAb (CLB-T3/4.E (final dilution ascites 1:1000)), in a final volume of 200 \(\mu\)l of the medium described above. Proliferation was measured as described above. Background proliferation of monocyte-depleted PBL cultured with CD3 mAb or allogenic PBMC alone was always <300 cpm.

2.7. Flow cytometry

After isolation, cells were washed twice with medium consisting of phosphate-buffered saline (PBS) supplemented with 0.5% bovine serum albumin (BSA). Immunofluorescence staining was performed by incubation of 2 \(\times 10^5\) PBMC during 30 min at 4°C with saturating amounts of combinations of PE- and FITC-labelled mAb in PBS/BSA. Stained cells were washed twice and resuspended in 200 \(\mu\)l PBS/BSA and 10\(^4\) viable lymphocytes were analyzed using a fluorescence activated cell sorter (FACS, Becton Dickinson, Sunnyvale, CA). Monocytes and non-viable lymphocytes were excluded from analysis on the basis of their forward/side-angle light scatter signal. Data are represented as the percentage with FITC-positive phenotype of CD3\(^+\), CD4\(^+\) or CD8\(^+\) cells.

2.8. Statistical analysis

Using the Kolmogorov-Smirnov test for goodness of fit, FACS-analysis data were found to be normally distributed. Therefore, comparison of these data between different groups was performed using Student's \(t\)-test. Because the data of functional assays were not found to be normally distributed, comparison between groups with regard to functional parameters was performed using the Mann-Whitney U-test.

3. Results

3.1. Diminished accessory cell-dependent responsiveness of Tcells from MS patients in whole blood cultures

Fig. 1 shows the results of the in vitro proliferation assays as measured in whole blood cultures (data are given as median cpm of 10 \(\mu\)l of blood). As can be seen here, there is a significant reduction in the ability of circulating T cells of MS patients to respond in these assays. After stimulation with soluble CD3 mAb, median cpm in healthy controls was 3546 (range 605–
11012). MS patients showed a markedly lower response (1504, range 122-11061, $P = 0.002$) upon this stimulus. Additionally, after stimulation with CD2 antibodies there was a significant reduction of the proliferative response of patients' T cells compared to healthy controls (Controls 5690, range 752-65996; MS 1653, range 159-8731; $P = 0.001$).

3.2. Responsiveness of PB $T$ cells from MS patients is not different from healthy controls in accessory cell-independent systems in standard cultures

Although the difference was less distinct than in the whole blood lymphocyte culture, MS patients also showed a lower response to soluble CD3 mAb in cultures of isolated PBMC as compared with healthy controls (Fig. 2A: median cpm controls 3017, range 104-25 960; MS 1764, range 105-31 686; $P = 0.023$). In contrast with the results of the whole blood cultures, no significant differences could be found in the proliferative response of peripheral blood mononuclear cells to a triplet of CD2 mAb (Fig. 2B: median cpm controls 7063, range 201-61 940; MS 15175, range 148-67 480).

PB $T$ cells from MS patients responded equally well compared to those from healthy controls after stimulation with immobilized CD3 mAb (Fig. 2C: median cpm controls 55 861, range 45 45-99 268; MS 45 373, range 47 37-83 045).

Using a combination of CD28 mAb and a triplet of CD2 mAbs, an enhanced proliferative response of
immune-mediated diseases. In asymptomatic men infected with human immunodeficiency virus-1 (HIV-1), the response to soluble CD3 mAb (IgE) in whole blood lymphocyte culture (Schellekens et al., 1990) was decreased in comparison with healthy controls. Furthermore, it has been demonstrated that PBMC of systemic lupus erythematosus (SLE) patients respond relatively poorly to Leu-4 (IgG1 mAb to CD3) (Kaneoka, 1992). In addition, PBMC of SLE patients seem to have an impaired response to CD2 mAb (Fox et al., 1991). The latter has recently also been demonstrated for chronic progressive (CP) MS patients (Reder et al., 1991).

In vitro proliferative responses of peripheral blood T cells can be measured relatively easy in whole blood cultures in a reproducible manner (Bloemena et al., 1989). Using this assay, we here show that the proliferative response of MS peripheral blood T cells after stimulation with CD3 and CD2 mAb is significantly reduced compared to healthy controls. In standard cultures, the response to soluble CD3 mAb was also significantly lower in MS patients. In contrast, after stimulation with immobilized CD3 mAb, and after stimulation with CD3 or CD2 mAb in combination with CD28 mAb in standard cultures, no differences were found in PB T cell proliferative responses between controls and MS patients. Differences between MS patients and healthy controls were not due to the unequal sex distribution between the two groups, because there were no significant differences in proliferative responses between males and females (data not shown). Also, we could exclude the possibility of lymphocytotoxic activity in MS sera as was found by others (Scott and Spiteri, 1983), on the basis of experiments in which we added sera of MS patients to PBMC of healthy donors, stimulated with soluble CD3 mAb. Under these conditions, T cell responses did not show any difference to those measured after the addition of healthy donor serum (data not shown). Furthermore, there was no difference in the number of cells per standard volume of blood between MS patients and controls (data not shown). Therefore, it can be concluded that MS patients show an impaired T cell response in accessory cell-dependent activation assays. The question arises what the underlying cause(s) may be of this in vitro functional defect. It could be that there is an inherent defect in monocytes of MS patients. It has been shown that in active MS the expression of HLA-DR antigens on monocytes is decreased (Baxevanis et al., 1989). However, this did not seem to be the result of a diminished monocyte responsiveness, because stimulation with interferon-γ and with LPS-enhanced the HLA-DR expression in stable and active MS equally well (Ransohoff et al., 1992). In our study we could find no evidence for a diminished capacity of MS monocytes to deliver co-stimulatory signals to T cells, because the response of healthy donor T cells in our system was equally well in the presence of irradiated PBMC either from MS patients or from healthy controls. While evidence for a defect in accessory cells in MS is apparently not very convincing, most of the studies seem to point to disturbances in T-cell function (Neighbour and Bloom, 1979; Antel et al., 1986; Hirsch, 1986; Chofflon et al., 1988). It has been shown that MS patients have impaired suppressor activity (Antel and Arnason, 1979). In subsequent studies it was postulated that this might be due to a defect in the CD8+ subpopulation (Antel et al., 1986). However, others have suggested that the diminished suppressor activity might be a result of a reduction in the percentage of CD4+CD45RA+ (suppressor inducer) subset (Chofflon et al., 1988). Also, a reduction in CD4+-mediated CTL activity specific for measles and herpes virus has been reported in MS patients (de Silva and McFarland, 1991). At the moment it is unclear whether persistent immune activation could account for these in vitro T cell stimulation defects. Furthermore, it remains to be investigated, using suboptimal stimulation conditions in accessory cell-independent activation systems, if PB T-cell function of MS patients still appears not to be any different from those of healthy controls.

On the basis of the results of the functional assays one might expect phenotypical changes in PB T cell subsets in MS patients, indicative of systemic immune activation. In the literature there seems to be little agreement about this. In some studies it has been demonstrated that especially chronic progressive MS patients and patients in relapse, show decreased numbers of CD4+CD45RA+ (unprimed) cells, formerly named suppressor inducer T cells (Rose et al., 1985; Morimoto et al., 1987; Porrini et al., 1992). In other studies, the decrease in CD4+CD45RA+ numbers could not be confirmed (Chofflon et al., 1989; Salonen et al., 1989). In contrast with the above mentioned reports, MS patients in our study showed a significantly higher percentage of CD4+CD45RA+ cells (50.1% ± 18.8%) when compared to healthy controls (38.6% ± 11.1%, P = 0.009). It is known that the percentage of CD4+CD45RA+ cells decreases with age (Serra et al., 1988). However, in our study the mean age of patients and controls was not significantly different (data not shown). An alternative explanation may be that there could be a selective migration of 'memory' effector cells to the CNS. This might also explain the fact that MS patients did not show enhanced numbers of circulating CD27- 'effector' cells (De Jong et al., 1992). Finally, we found no evidence for a differential expression of the T cell activation marker CD25 in peripheral blood from mixed types of MS patients. This seems to be in contrast with the finding that in peripheral blood from progressive MS patients the expression of CD26 (Ta+) is enhanced compared to patients having stable or improving multiple sclerosis, patients with other
neurological diseases, and normal controls (Hafler et al., 1985a).

Altogether, on the basis of the phenotypical analyses, this study provides no evidence for gross changes in PB T cell activation and/or differentiation in MS patients. However, using functional assays, in accessory cell-dependent systems, patients showed a markedly lower response of peripheral blood T cells as compared with healthy controls. In contrast to CD4+CD45RO+ cells, CD4+CD45RA+ cells have suppressor-inducer capacity (Morimoto et al., 1985) and appear to have more strict requirements for proliferation induction (Byrne et al., 1988, 1989; Sanders et al., 1989). Because MS patients show higher numbers of these cells, one might expect this to play a role in the in vitro functional defects observed. However, in our study no correlation was found at an individual basis between numbers of CD4+CD45RA+ cells and in vitro proliferative T cell responses (data not shown). In addition, as reviewed by Westermann and Pabst (1990), one needs to be cautious with the interpretation of lymphocyte subset changes in blood in relation to pathological changes in other organs, and with the use of phenotypical markers as a reflection of functional lymphocyte subpopulations. Other factors, such as selection of patients, medication, and general condition of the patient, must also be taken into account. Therefore, further investigation is needed in order to clarify the relation between in vitro responsivity in the functional assays described here and the clinical condition of the patient on the one hand, and (local) disease processes on the other.

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