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Chronic Alcohol Consumption Is Associated With Changes in the Distribution, Immunophenotype, and the Inflammatory Cytokine Secretion Profile of Circulating Dendritic Cells

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Abstract

Background: Alcoholism is frequently associated with altered immune responses, limited information being available on its effects on dendritic cells (DC). In the present study we analyze the effects of chronic alcoholism on circulating DC.

Methods: For the first time we studied the numerical distribution of DC in peripheral blood (PB), their immunophenotype, and their ex vivo pattern of spontaneous cytokine secretion, in chronic alcoholic patients without liver disease (AWLD group; $n=17$) and active ethanol (EtOH) intake, as well as in subjects with alcohol liver cirrhosis (ALC group; $n=21$).

Results: A significantly decreased HLADR expression and an increased reactivity for CD123 was observed on PB DC from AWLD patients; additionally, increased secretion of interleukin (IL) 1 β , IL6, IL12, and tumor necrosis factor- α (TNF α) by DC was also noted in this group. Conversely, patients with ALC and at least 1 year of alcohol withdrawal (ALCAW group) showed a decreased number of total circulating DC, whereas ALC patients with active EtOH intake (ALCET group) had an abnormally low production of IL1 β and TNF α by PB DC.

Conclusion: Chronic alcoholism in the absence of liver disease is associated with an increased secretion of inflammatory cytokines by PB DC, whereas ALCAW and ALCET patients show decreased numbers of circulating DC and reduced secretion of these cytokines, respectively. IT IS WELL KNOWN that alcoholism is associated with an imbalanced immune response, the exact abnormalities detected being related to both the status of ethanol (EtOH) intake and the existence of alcohol liver disease (ALD) ([Laso et al., 1996a, 1996b, 1997a, 1997b](#)). Accordingly, alcoholic individuals frequently show an immunodeficiency state which is associated with a higher risk of suffering from infection and cancer ([McGregor, 1986; Nelson and Jolls, 2002; Szabo, 1999](#)). In line with this, acute alcohol administration has been shown to reduce antigen presentation by monocytic cells ([Szabo et al., 2004](#)) and to suppress secretion of inflammatory cytokines ([Mandrekar et al., 2004; Szabo et al., 1996, 1999; Verma et al., 1993](#)) through toll-like receptors (TLR) ([Pruett et al., 2004](#)). Conversely, chronic alcoholism has been related to a T-cell activation, particularly at the expense of CD8⁺ cytotoxic T-lymphocytes ([Cook et al., 1991, 2004; Jerrells, 2002; Kronfol et al., 1993; Laso et al., 1996a, 1996b, 1997b, 2000; Santos-Perez et al., 1996; Song et al., 2002](#)), a higher secretion of monocyte-derived inflammatory cytokines ([Bode and Bode, 2005; McClain et al., 1999](#)) and a greater expression of the CD80 and CD86 costimulatory molecules on macrophages ([Cook et al., 2004; Zhu et al., 2004](#)).

Dendritic cells (DC) are the most efficient antigen presenting cells (APC) to naive T-lymphocytes, playing a crucial role on innate and adaptive immune responses ([McKenna et al., 2005; Mellman and Steinman, 2001](#)). In recent years, 2 major subsets of DC have been well characterized in peripheral blood (PB): the myeloid (mDC) ([Mellman and Steinman, 2001](#)) and plasmacytoid DC (pDC) ([McKenna et al., 2005](#)). In addition, a third subset of APC has been identified in normal PB by our group and other authors ([Almeida et al., 2001; Grage-Griebenow et al., 2001; Schakel et al., 1998](#)). These APC show reactivity for the M-DC8 monoclonal antibody (MAb) clone ([Schakel et al., 1998](#)), they display high expression of Fc γ RIII (CD16)—a marker absent in the other 2 PB DC subsets—and they are positive for DC-SIGN (CD209)—a C-type lectin exclusively expressed on APC; from the functional point of view, M-CD8⁺/CD16⁺DC show an important stimulatory capacity for naive T cells in mixed allogenic cell cultures ([Rivas-Carvalho et al., 2004](#)). Recent studies suggest that acute alcohol consumption could down-regulate antigen presentation and expression of the CD80 and CD86 costimulatory molecules on DC ([Szabo et al., 2004](#)). However, no studies have been reported so far in which the phenotypic and functional characteristics of the different subsets of PB DC from individuals suffering from chronic alcoholism are analyzed. This is particularly true because accumulating evidences exist about the lack of relationship between the immunological effects of acute and chronic alcoholism. In the present study, we analyzed for the first time the distribution as well as the immunophenotypic and functional characteristics of PB DC from patients with chronic alcoholism, grouped according to their status of EtOH intake, and the presence or absence of alcohol liver cirrhosis (ALC) at the moment of the study. Our results indicate that chronic alcoholism is associated with numerical, phenotypical, and functional changes in circulating PB DC, consisting of an increased secretion of inflammatory cytokines in chronic alcoholic patients without liver disease and on decreased numbers of circulating DC and reduced secretion of the same cytokines among ALC patients with at least 1 year of alcohol withdrawal (ALCAW group) and ALC patients with active EtOH intake (ALCET group), respectively.

MATERIALS AND METHODS

Patients, Control Subjects, and Samples

A total of 38 alcoholic male patients referred to the Alcoholism Unit of the University Hospital of Salamanca were included in this study. From them, 17 were alcoholic patients without liver disease (AWLD group) and 21 patients were diagnosed as having ALC. The most relevant clinical and biological data about both groups of patients at the time of entering the study are shown in [Table 1](#). In parallel to the patients, 10 healthy male volunteers (control subjects) were also analyzed. Before entering the study, all individuals (patients and controls) gave their informed consent to participate, and the study was approved by the Ethics Committee of the University Hospital of Salamanca. For each case, EDTA and heparin anticoagulated PB samples were obtained between 9:00 and 10:00 am under fasting conditions.

AWLD Group ($n=17$). All patients included in this group were actively drinking (≥ 90 g of EtOH/day) until the day of entering the study. Subjects who were polydrug abusers and those displaying physical stigmata of chronic liver disease (i.e., cutaneous signs, hepatosplenomegaly, gynecomastia, testicular atrophy, and/or muscle wasting), were excluded from the study. All patients had normal hemoglobin concentrations, prothrombin time, and albumin serum levels. Alanine aminotransferase and aspartate aminotransferase serum levels were required to be less than twice the upper normal limits (40 IU/L). Hepatitis B surface antigen (HbsAg) and antibodies to hepatitis C virus (HCV) and to human immunodeficiency virus (HIV) were constantly negative. Liver biopsy was not performed for ethical reasons, liver disease being excluded on the basis of ultrasonographic studies. To avoid enrolling patients with malnutrition, evaluation of the nutritional status was made using both laboratory parameters (serum albumin concentration and transferrin levels) and anthropometric analyses (height, weight, triceps skin-fold, and midarm muscle circumference measurements); the results were compared with published criteria to derive percentages of standard values ([Bishop et al., 1981](#)). Only subjects with anthropometric tests $>90\%$ of expected normal values were included in the study.

Alcoholic Patients With Liver Cirrhosis ($n=21$). Depending on their status of alcoholic intake at the time of entering the study, this group of patients was subdivided into 2 categories: (1) patients with ALC and at least 1 year of alcohol withdrawal (ALCAW group; $n=10$); and (2) patients with ALC and active EtOH intake at the moment of entering the study (ALCET group; $n=11$). Histopathological examination of the liver in all 14 cirrhotic patients in whom it was performed revealed micronodular cirrhosis, but no signs of alcoholic hepatitis. In the remaining 7 patients, liver biopsy was not performed because of blood coagulation abnormalities. In these cases, diagnosis of ALC was established on the basis of the presence of physical stigmata of chronic liver disease, history of ascites, variceal bleeding, or hepatic encephalopathy, as well as on gastroscopic and/or ultrasonographic findings ([Conn and Atterbury, 1987](#)). All patients were HBsAg (–), HCV antibodies (–), and HIV antibodies (–), and none of them had malnutrition according to the above-mentioned criteria. None of the ALC patients had been diagnosed as having acute alcoholic hepatitis before the time of entering the study. None had ascites, jaundice, encephalopathy, or gastrointestinal bleeding in the year before the study. None of them had received blood transfusions or was under treatment with steroids or immunosuppressive therapy.

Control Subjects ($n=10$). All control subjects reported to drink <15 g of EtOH/day. Liver function tests, as well as routine hematological and biochemical tests, were within the normal ranges.

Enumeration and Immunophenotypic Characterization of DC Subsets

Whole PB samples (approximately 1×10^6 cells in $100 \mu\text{L}$ /test) were stained by a direct immunofluorescence technique using 4-color combinations of MAb directly conjugated with the following fluorochromes: fluorescein isothiocyanate (FITC), phycoerythrin (PE), peridinin chlorophyll protein (PerCP), and allophycocyanin (APCy). In all tests, a mixture of FITC-

conjugated MAb directed against T lymphocytes, B lymphocytes, natural killer cells, and monocytes was used at saturating concentrations: CD3-FITC [Leu-4; Becton Dickinson Biosciences –(BDB), San Jose, CA], CD19-FITC (Leu-12; BDB), CD56-FITC (clone C5.9; IMICO, Madrid, Spain), and CD14-FITC (Leu-M3; BDB), respectively. In addition, CD33-PE (Leu-M9; BDB), anti-HLADR-PerCP (clone L243; BDB), and CD123-APC (clone 9F5; PharMingen, San Diego, CA) were also added at saturating concentrations. Samples were then incubated for 15 minutes at room temperature in the dark. Afterwards, 2 mL of FACS lysing solution (BDB) diluted 1:10 (v/v) were added and the samples were incubated for another 10 minutes under the same conditions described above. After 2 washes with phosphate-buffered saline (PBS; pH=7.4), samples were resuspended in 0.5 mL of PBS/tube. Data acquisition was performed in 2 consecutive steps in a FACSCalibur flow cytometer, using the CellQUEST software (BDB): in the first step, information about 3×10^4 events/test, corresponding to the whole PB cellularity, was stored. In the second step, information on a minimum of 3×10^5 HLADR+ cells was collected. For data analysis, the Paint-A-Gate PRO software (BDB) was used. For each antigen analyzed, the intensity of expression was evaluated by mean fluorescence intensity (MFI) expressed as arbitrary relative linear fluorescence units, scaled from 0 to 10^4 .

Ex Vivo Analysis of Cytokine Production by PB DC

Spontaneous ex vivo cytokine production by PB DC was analyzed at the single cell level using a technique that combines 4-color stainings for the specific identification of the different DC populations, and the measurement of cytokine production on erythrocyte-lysed whole blood samples ([Almeida et al., 2005](#)). Briefly, 500 μ L of heparin-anticoagulated PB diluted 1:1 in RPMI 1640 (BioWhittaker, Walkersville, MD) supplemented with 2 mM l-glutamine was placed in a tube containing 10 μ g/mL of brefeldin A (BFA; Sigma, St. Louis, MO) to block cytokine secretion, in the absence of any exogenous stimuli. Afterwards, cells were incubated for 6 hours at 37°C in a 5% CO₂ and 95% humidity, sterile environment. Once this incubation period was completed, the sample was aliquoted in different tubes and stained with 10 μ L of CD3-FITC, CD14-FITC, CD19-FITC, CD56-FITC, anti-HLADR-PerCP, and 5 μ L of CD33-APC (Leu-M9; BDB), as described above. Then, cells were fixed, permeabilized, and stained with MAb directed against different human cytokines, using the Fix & Perm reagent (Caltag Laboratories, San Francisco, CA), strictly following the manufacturers' recommendations. The source and specificities of the MAb used to detect intracytoplasmic human cytokines were as follows: anti-tumor necrosis factor- α (TNF α)-PE (clone Mab 11; PharMingen), anti-interleukin (IL) 6-PE (clone MQ2-6A3; PharMingen), anti-IL12-PE (clone C11.5; PharMingen), and anti-IL1 β (clone AS10; BDB). Data acquisition and analysis were performed in a FACSCalibur flow cytometer, as described above. Evaluation of cytokine production was based on both the percentage of positive cells and their MFI within each of the cell populations under study.

Analysis of Soluble Cytokine Plasma Levels

Plasma was collected from PB samples and immediately stored at –80°C until further processed. Soluble plasma levels of both interferon- α (IFN α) and IL3 were measured using commercially available ELISA kits: human interferon alpha serum sample ELISA kit (PBL Biomedical Laboratories, Piscataway, NJ; lower sensitivity level for the high sensitivity protocol: 10 pg/mL) and Quantikine Human IL3 Immunoassay kit (R&D, Abingdon, U.K.; lower sensitivity level: 30 pg/mL). ELISA plates were read using the ULTRA EVOLUTION Multifunctional Reader (Tecan; Toronto, Canada) at a 450 nm excitation wavelength.

Statistical Analysis

Mean values, standard deviations, median and range, as well as the 25th and 75th percentiles, were calculated for each variable under study using the SPSS software program (SPSS 12.0, Chicago, IL). The Mann–Whitney *U* and Kruskal–Wallis nonparametric tests were used for the

evaluation of the statistical significance of the differences observed between 2 or more groups, respectively. p -Values <0.05 were considered to be associated with statistical significance.

RESULTS

Distribution and Immunophenotypic Characteristics of PB DC

Three different subsets of DC, corresponding to mDC, pDC, and CD16+DC, were found in the PB of all individuals from both the patient and control groups ([Fig. 1](#), upper panels). The 3 PB DC subsets were identified according to their differential expression of CD33, as previously reported ([Almeida et al., 2001, 2005](#)); accordingly, within the whole population of PB DC (identified as those cells that lacked CD3, CD14^{hi}, CD19, and CD56 and showed strong reactivity for HLA-DR; [Fig. 1A](#)), mDC were identified by their high expression of CD33 and HLA-DR ([Fig. 1B](#)), pDC were identified as CD33^{lo}/HLA-DR^{+/++} events (as cells identified by this way were shown to express high levels of CD123, considered to be highly specific for pDC; [Fig. 1C](#)) and CD16+ DC were identified by their intermediate levels of CD33 in the presence of lower levels of HLA-DR ([Fig. 1D](#)). Overall, alcoholic patients showed normal numbers of DC in PB ([Table 2](#)). Nevertheless, a more detailed analysis of the different groups of patients showed that, while AWLD patients had normal numbers of all PB DC subsets, ALC patients with at least 1 year of alcohol withdrawal (ALCAW group) displayed a significantly decreased overall number of circulating DC ($p<0.05$) with respect to healthy controls, due to a selective decrease of both mDC ($p<0.05$) and CD16+ DC ($p<0.05$) ([Table 2](#)). Alcohol liver cirrhosis patients with active EtOH intake displayed a slightly lower number of total PB DC, due to a decrease of circulating CD16+ DC ($p<0.05$) ([Table 2](#)).

Detailed analysis of the immunophenotypic characteristics of PB DC from the different groups of patients analyzed showed significant differences between them as well as with respect to the control subjects ([Fig. 2](#)). Accordingly, AWLD patients showed a significantly decreased HLA-DR expression in all different subsets of circulating DC with respect to ALCET patients; in addition, HLA-DR expression on CD16+ DC from the AWLD group was also significantly decreased with respect to healthy controls. In turn, expression of CD123 on both mDC and CD16+DC, but not pDC, from AWLD patients was significantly increased with respect to both controls and ALCET patients ($p<0.05$). In contrast, CD33 was expressed at normal levels in AWLD patients in all 3 subsets of DC analyzed.

Among cirrhotic patients, DC from those individuals included in the ALCAW group showed a normal phenotype, except for an abnormally decreased reactivity for CD33 on pDC with respect to controls. Regarding the ALCET group, these patients showed a significantly lower ($p<0.05$) CD123 and CD33 expression than healthy subjects on both mDC and CD16+ DC, but not pDC, together with normal HLA-DR expression on the 3 subsets of DC analyzed.

Spontaneous Secretion of Inflammatory Cytokines by PB DC

Overall, detectable amounts of spontaneously secreted inflammatory cytokines were found in circulating PB mDC, CD16+ DC, and pDC from chronic alcoholic patients, as illustrated in [Fig. 1](#) (lower panels). Compared with control individuals, only mDC and CD16+ DC showed significant changes in the secreted amounts of these cytokines ([Fig. 3](#)). Accordingly, in AWLD patients both PB DC subsets showed higher secretion of IL1 β and TNF α ($p<0.05$ in both cases) in comparison with the controls and the other 2 patient groups; additionally, an increased secretion of IL12 by mDC ($p<0.05$) and CD16+ DC ($p<0.05$) was also noted in the AWLD group with respect to the 2 ALC patient groups. Regarding secretion of IL6, mDC and CD16+ DC from AWLD patients also showed an increased production of this cytokine *ex vivo* with respect to both normal controls ($p<0.05$) and ALCET patients ($p<0.05$). Of note, AWLD patients displayed increased plasma IL3 levels with respect to both the controls and ALCAW patients ($p<0.05$; [Fig. 4](#)), in the absence of detectable IFN α plasma levels: median levels of 0 pg/mL

(range 0–29 pg/mL), 0 pg/mL (range 0–28 pg/mL), 0.5 (range 0–210 pg/mL) and 0 pg/mL (range 0–0 pg/mL) in controls, AWLD, ALCAW, and ALCET patients, respectively ($p>0.05$).

Alcohol liver cirrhosis patients with active EtOH intake showed an abnormally low ex vivo production of the IL1 β and TNF α inflammatory cytokines by mDC ($p<0.05$) as well as of IL β by CD16+ DC ($p<0.05$), with respect to both controls and ALCAW group of patients ([Fig. 3](#)). No statistically significant differences were found in the secretion of IL1 β , IL6, IL12, and TNF α once ALCAW patients were compared with controls.

DISCUSSION

Accumulating evidences indicate that alcoholism induces alterations of the immune response. Although the most frequently reported immunological finding is an immunodeficiency state ([McGregor, 1986](#); [Nelson and Jolls, 2002](#); [Szabo, 1999](#)), a paradoxical activation of cytotoxic T and NK cells ([Cook et al., 1991, 2004](#); [Jerrells, 2002](#); [Kronfol et al., 1993](#); [Laso et al., 1996a, 1996b, 1997a, 1997b, 2000](#); [Santos-Perez et al., 1996](#); [Song et al., 2002](#)) as well as of macrophages ([Zhu et al., 2004](#)) has been consistently reported in the literature in chronic alcoholism. In contrast no study has been reported so far in which the specific phenotypic and functional characteristics of the different subsets of DC circulating in the PB of chronic alcoholism patients has been systematically analyzed. In the present study we analyzed the numerical distribution of the 3 subsets of PB DC, as well as their immunophenotypic changes and their pattern of spontaneous ex vivo secretion of inflammatory cytokines.

Our results show that AWLD patients have normal numbers of mDC, pDC, and CD16+ DC in PB, associated with a more immature phenotype of all 3 subsets of DC, as reflected by a significantly decreased expression of the HLADR antigen-presenting class II molecule ([Mellman and Steinman, 2001](#)). At present it is well established that EtOH increases intestinal permeability, favoring bacterial translocation ([Bode et al., 2001](#); [Mathurin et al., 2000](#); [Parlesak et al., 2000](#)). The entrance of endotoxin [lipopolysaccharide (LPS)] into the circulation would trigger activation of DC at the gut-associated lymphoid tissue (GALT). Thus, it could be hypothesized that the immature phenotype of PB DC from AWLD patients could be related with an increased turnover of DC following overstimulation and activation of intestinal DC by LPS during chronic EtOH intake, leading to an early release of less mature DC from the bone marrow into PB; such changes would not be evident in ALC patients, due to a decreased entrance of newly produced DC into the PB, as discussed below.

Regarding expression of CD123 (IL3 receptor α -chain) on PB DC from AWLD patients, our results show a significant increase in both mDC and CD16+ DC, but not pDC, the exact mechanism being unknown. Interestingly, plasma levels of soluble IL3 (which is mostly produced by mature T-lymphocytes and to a lower extent mast cells; [Frendl, 1992](#)), were specifically increased in this patient group (AWLD), further studies being required to investigate the potential biological and clinical significance of these findings.

In line with the hypothesis about the existence of an increased in vivo activation of DC in chronic alcoholism, PB mDC and CD16+ DC from AWLD patients showed significantly increased spontaneous ex vivo secretion of IL1 β , IL6, IL12, and TNF α . To the best of our knowledge, no studies have been reported so far in which secretion of these cytokines by PB DC has been analyzed in chronic alcoholism. Despite this, it has been shown that acute alcohol treatment inhibits secretion of IL1 β , IL6, and TNF α by human PB monocytes ([Szabo et al., 1996, 1999](#); [Verma et al., 1993](#)). Interestingly, while spontaneous ex vivo secretion of IL12 was found to be increased among AWLD patients with respect to the other ALC patient groups, no differences were observed on the levels of this cytokine in comparison with the control group; these latter findings would be in contrast with the previously reported in vitro and in vivo inhibitory effect of acute alcohol administration on the secretion of this cytokine by DC ([Mandrekar et al., 2004](#)). Secretion of inflammatory cytokines by DC depends on activation of nuclear factor κ B, which is up-regulated, among other conditions, through the interaction of LPS

with TLR-4 on the cell surface ([Kawai and Akira, 2005](#)). Thus, while acute exposure to EtOH inhibits TLR signaling by LPS ([Mandrekar et al., 2002](#); [Pruett et al., 2004](#)), decreasing the synthesis of inflammatory cytokines, chronic alcohol feeding has been shown to increase cellular sensitivity to the effects of LPS in mice ([Romics et al., 2005](#)), which would lead to secretion of these cytokines. In addition, the increased secretion of IL12 released by DC under stimulation with LPS primes a T-helper (Th)-1 response ([de Jong et al., 2005](#)), supporting the notion that active EtOH intake in patients without liver injury is associated with a predominant Th-1 pattern of intracytoplasmic cytokine secretion by T cells ([Laso et al., 1999](#)). Moreover, both IL-12 and the Th-1 cytokines favor activation and expansion of effector CD8⁺ cytotoxic T-lymphocytes, a T-cell subset that could play a role in the development and outcome of ALD ([Jerrells, 2002](#)). Alcohol liver cirrhosis patients with at least 1 year of alcohol withdrawal showed decreased numbers of total circulating DC, due to a selective reduction of both mDC and CD16⁺ DC. Such observation could be due to: (1) an increased migration and compartmentalization of DC to the liver, as recently suggested in nonalcoholic chronic liver injury ([Kunitani et al., 2002](#); [Ulsenheimer et al., 2005](#); [Wertheimer et al., 2004](#)), where they would contribute to ALD; (2) trapping and destruction of circulating DC in the spleen, as it happens with other white blood cells and platelets in patients with advanced chronic liver disease ([Peck-Radosavljevic, 2001](#)); and (3) decreased bone marrow output of DC. Moreover, previous studies have shown significantly increased numbers of CD4⁺/CD25⁺T regulatory lymphocytes in the circulation of patients with ALD ([Laso et al., 1997b](#)), which may exert a suppressive effect on DC ([Pasare and Medzhitov, 2003](#)) and could contribute to explain the lower secretion of inflammatory cytokines by PB DC of ALCET patients. Interestingly, ALCET patients displayed an abnormally low expression of CD123 on both mDC and CD16⁺ DC. Low expression of CD123 on DC has also been reported in autoimmune liver disorders ([Hiasa et al., 2002](#)), its exact significance remaining unknown. Altogether, our findings point out to the potential existence of a increased homing of myeloid and CD16⁺ DC into the GALT, associated in AWLD individuals, but not ALC patients, with an increased turnover of PB circulating immature DC expressing higher levels of both HLADR and CD123; in addition, chronic EtOH consumption in the absence of ALD would also lead to an increased in vivo secretion of IL1, IL6, and TNF α , while production of these inflammatory cytokines is significantly decreased in ALCET patients. Regardless of the mechanisms involved, the reduced number of circulating DC and/or the decreased secretion of inflammatory cytokines could lead to a subsequent immunodeficiency state, associated with a higher risk of infection.

In summary, our results show the existence of numerical, phenotypical, and functional changes in circulating PB DC in chronic alcoholism. Interestingly, active EtOH intake in the absence of liver disease is associated with increased secretion of inflammatory cytokines by DC, whereas ALCAW and ALCET patients showed decreased numbers of circulating DC and reduced secretion of these cytokines, respectively.

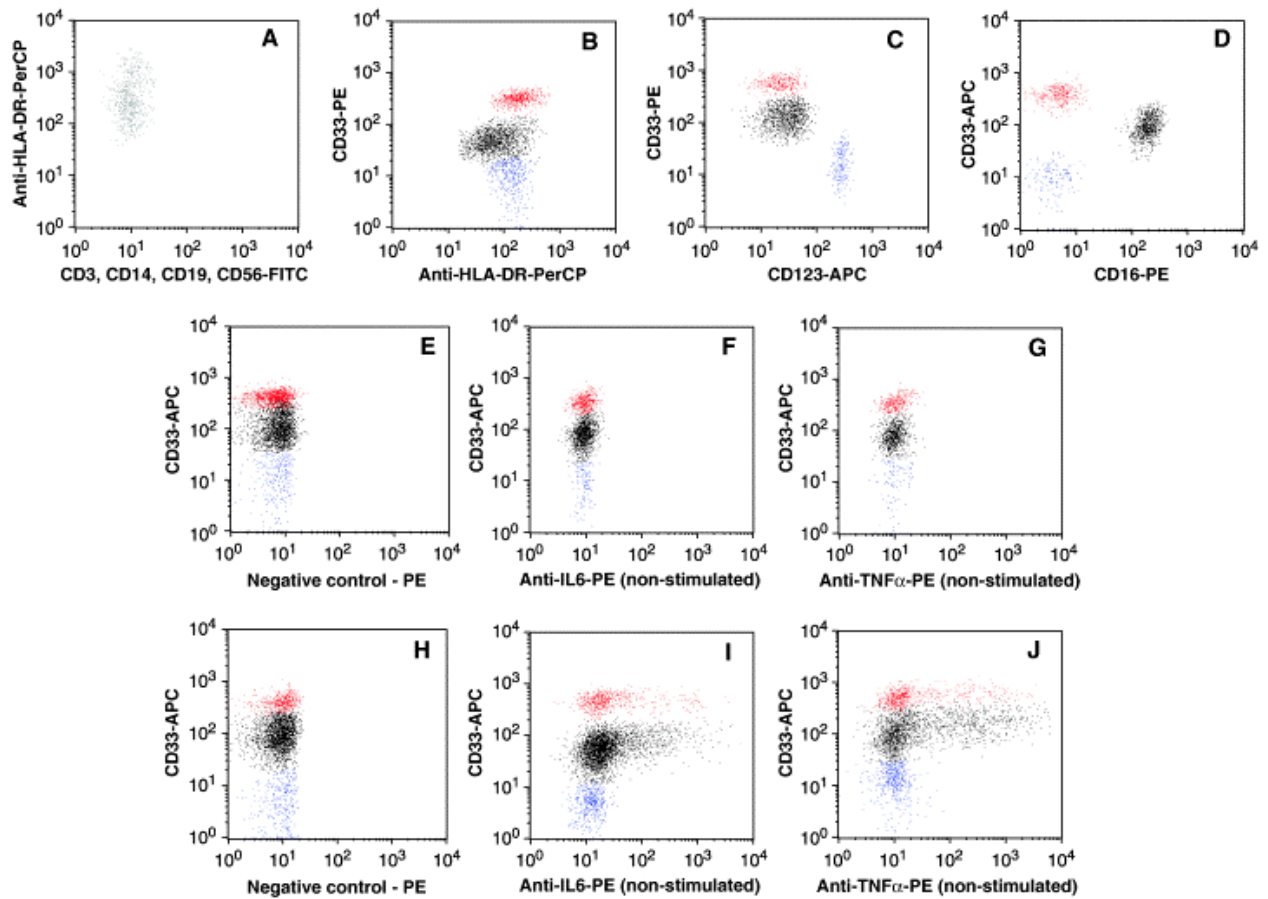


Fig. 1. Immunophenotypic identification (upper panels) and spontaneous cytokine production by different subsets of dendritic cells in peripheral blood samples from both healthy subjects (middle panels) and chronic alcoholic patients (lower panels). Total DC are included within the HLA-DR⁺⁺/Lin^{/lo} events (A). The 3 peripheral blood DC subsets (mDC, red dots; CD16⁺ DC, black dots; and pDC, blue dots) can be identified on the basis of their different reactivity for CD33 and HLA-DR (B): CD33^{/lo}/HLA-DR⁺⁺ DC correspond to pDC, confirmed by their high expression of CD123 (C) and CD33^{int}/HLA-DR⁺ DC correspond to CD16⁺ DC, verified by their positivity for CD16 (D). Illustrative examples of spontaneous ex vivo (nonstimulated) production of IL6 and TNF by PB DC subsets from 1 AWLD patient (I and J), in comparison with a representative healthy subject are displayed in (F) and (G). (E) and (H) show an isotype mIg-PE staining (negative control for PE conjugated antibodies) of DC subsets from the same control subject and 1 AWLD patient, respectively. DC, dendritic cells; mDC, myeloid DC; pDC, plasmacytoid DC; AWLD, alcoholic patient without liver disease; mIg-PE, mouse immunoglobulin-phycoerythrin; IL, interleukin; TNF α , tumor necrosis factor- α .

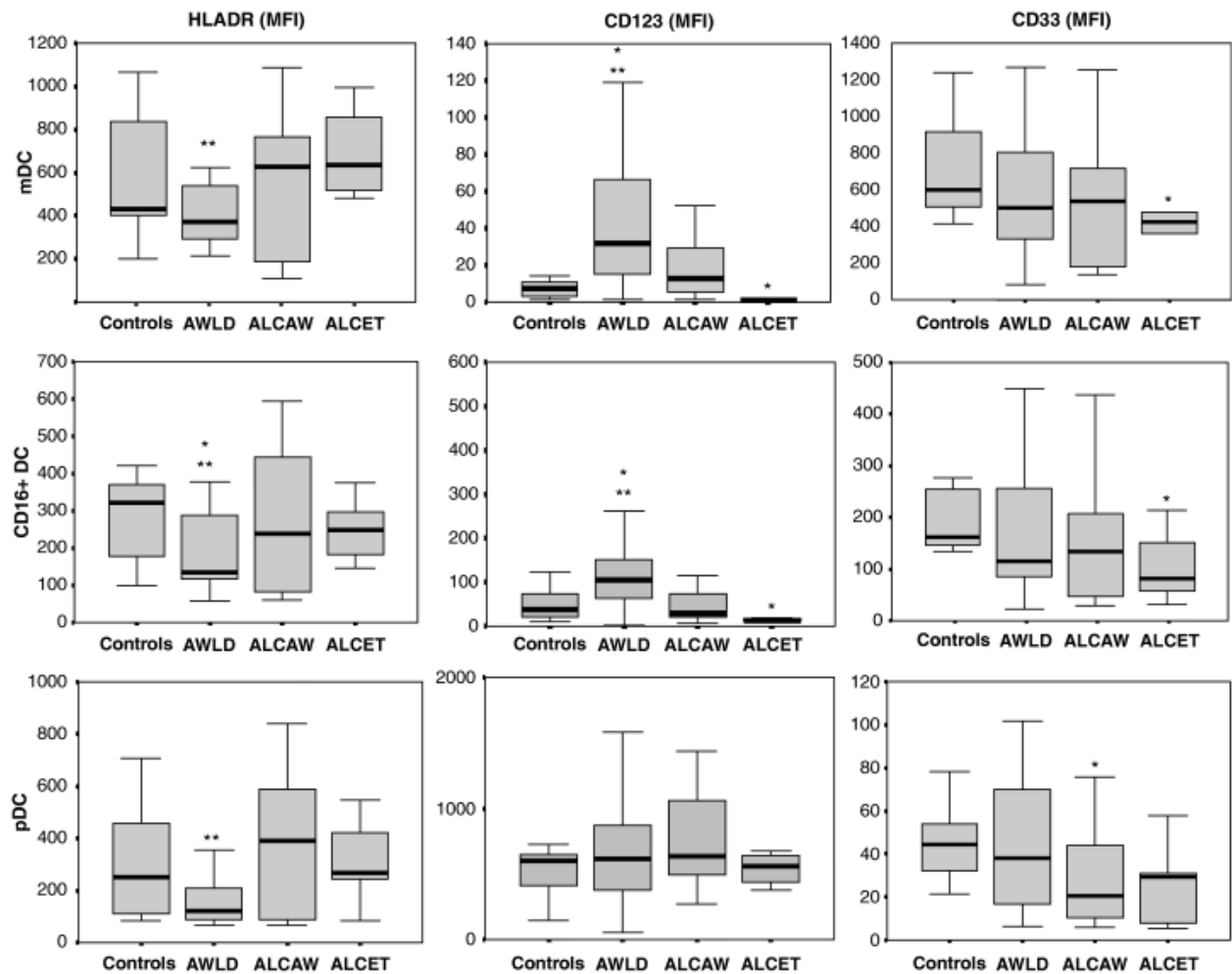


Fig. 2. Expression of CD33, HLADR, and CD123 by different subsets of dendritic cells in peripheral blood samples from chronic alcoholic patients and healthy individuals. Results are expressed as mean fluorescence intensity (MFI; arbitrary relative linear scaled from 0 to 10^4). Notched boxes represent 25th and 75th percentile values; the line in the middle and the vertical lines correspond to the median value and both the 10th and 90th percentiles, respectively. Baseline nonspecific staining intensities were similar among the different patient groups. *Statistically significantly different ($p < 0.05$) versus controls. **Statistically significantly different ($p < 0.05$) versus ALCET group. DC, dendritic cells; mDC, myeloid DC; pDC, plasmacytoid DC; AWLD, alcoholic patients without liver disease; ALCAW, alcoholic patients with liver cirrhosis and at least 1 year of alcohol withdrawal; ALCET, alcoholic patients with liver cirrhosis and active ethanol intake.

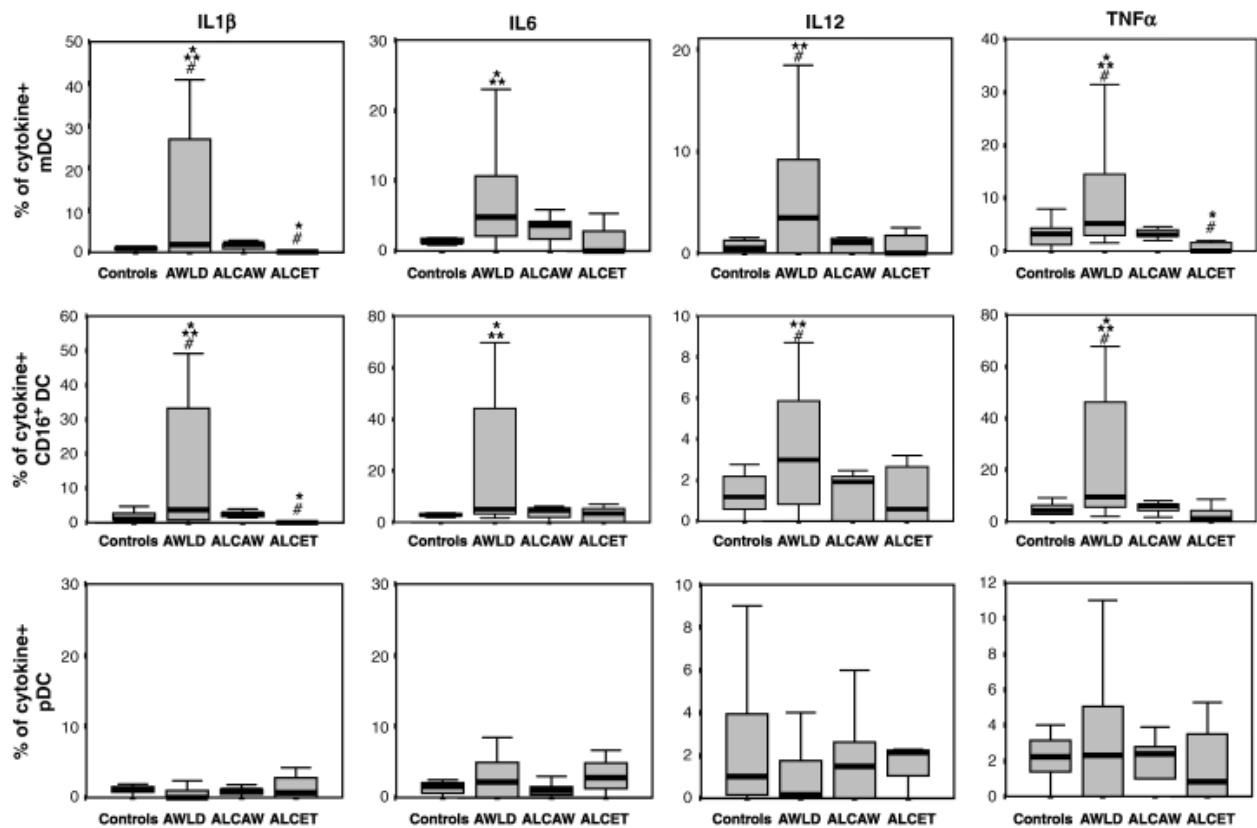


Fig. 3. Spontaneous ex vivo secretion of IL1 β , IL6, IL12, and TNF α by different subsets of dendritic cells in peripheral blood samples from chronic alcoholic patients. Results are expressed as percentage of cytokine-producing cells. Notched boxes represent 25th and 75th percentile values; the line in the middle and the vertical lines correspond to the median value and both the 10th and 90th percentiles, respectively. * Statistically significantly different ($p < 0.05$) versus controls. ** Statistically significantly different ($p < 0.05$) versus ALCET group. # Statistically significantly different ($p < 0.05$) versus ALCAW group. DC, dendritic cells; mDC, myeloid DC; pDC, plasmacytoid DC; IL, interleukin; TNF α , tumor necrosis factor- α ; AWLD, alcoholic patients without liver disease; ALCAW, alcoholic patients with liver cirrhosis and at least 1 year of alcohol withdrawal; ALCET, alcoholic patients with liver cirrhosis and active ethanol intake.

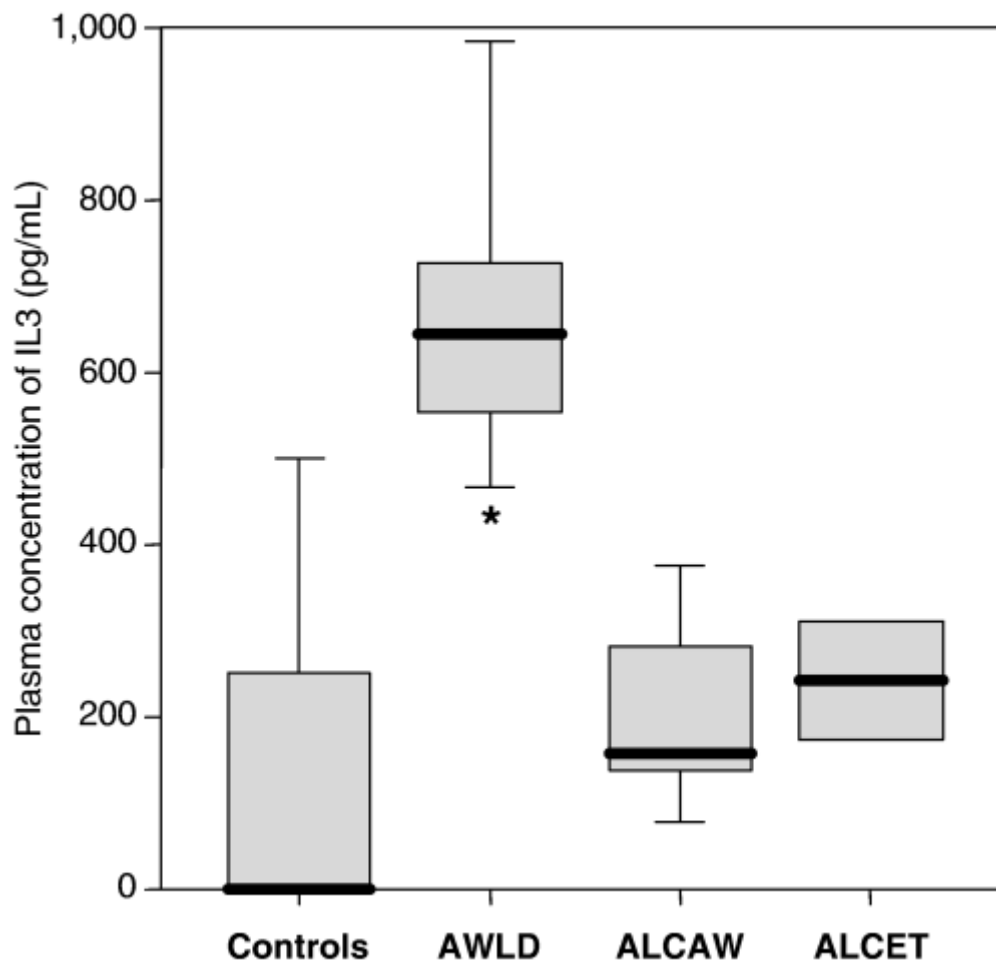


Fig 4. Plasma concentration of soluble IL3 in patients with chronic alcoholism and healthy individuals. Results are expressed as pg/mL of soluble IL3. Notched boxes represent 25th and 75th percentile values; the line in the middle and the vertical lines correspond to the median value and both the 10th and 90th percentiles, respectively. *Statistically significantly different ($p < 0.05$) versus both controls and ALCAW patients. IL, interleukin; AWLD, alcoholic patients without liver disease; ALCAW, alcoholic patients with liver cirrhosis and at least 1 year of alcohol withdrawal; ALCET, alcoholic patients with liver cirrhosis and active ethanol intake.

Table 1. Clinical and Biological Characteristics of Both Healthy Controls and Chronic Alcoholic Patients

	Controls (n=10)	AWLD (n=17)	ALCAW (n=10)	ALCET (n=11)
Mean age (y)	33 ± 6	49 ± 9	49 ± 8	54 ± 11
Bilirubin (0–1 mg/dL)	0.7 ± 0.1	0.9 ± 0.2	1.9 ± 0.2	2.1 ± 0.1
AST (1–40 IU/L)	23 ± 6	38 ± 9	54 ± 5	59 ± 4
GGT (9–61 IU/L)	26 ± 13	112 ± 13	67 ± 9	148 ± 21
INR (0.96–1.04)	1.01 ± 0.01	1.03 ± 0.01	1.23 ± 0.02	1.30 ± 0.02
Albumin (35–50 g/L)	51 ± 8	43 ± 7	35 ± 2	34 ± 3
Hemoglobin (13–17 g/dL)	15 ± 1	16 ± 1	13 ± 2	14 ± 2

Child–Pugh score (A/B)			10/0	8/3
Results expressed as mean $\pm$ 1 standard deviation. Normal ranges for the laboratory parameters are shown in brackets. AST, aspartate aminotransferase; GGT, γ -glutamyl transferase; INR, international normalized ratio; AWLD, alcoholics without liver disease; ALCAW, alcoholics with liver cirrhosis and at least 1 year of alcohol withdrawal; ALCET, alcoholics with liver cirrhosis and active ethanol intake.				

Table 2. Distribution of Total Leucocytes and Dendritic Cells in Peripheral Blood Samples From Chronic Alcoholic Patients and Healthy Individuals

	Controls (n=10)	AWLD (n=17)	ALCAW (n=10)	ALCET (n=11)
Leukocytes	6,298 $\pm$ 1,747	6,904 $\pm$ 1,804	5,666 $\pm$ 1,676	6,000 $\pm$ 2,091
Total DC	63 $\pm$ 25	65 $\pm$ 33	43 $\pm$ 15*	53 $\pm$ 26
mDC	10 $\pm$ 3	8.4 $\pm$ 5	7.1 $\pm$ 4*	9 $\pm$ 7
CD16+ DC	43 $\pm$ 25	43 $\pm$ 24	26 $\pm$ 10*	28 $\pm$ 14*
pDC	9 $\pm$ 3	8 $\pm$ 7	7 $\pm$ 3	14 $\pm$ 11
Results expressed as mean $\pm$ 1 standard deviation of the absolute number of each cell population/ μ L. *Statistically significantly different ($p < 0.05$) from controls. DC, dendritic cells; mDC, myeloid DC; pDC, plasmacytoid DC; AWLD, alcoholics without liver disease; ALCAW, alcoholics with liver cirrhosis and at least 1 year of alcohol withdrawal; ALCET, alcoholics with liver cirrhosis and active ethanol intake.				

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