Clin Exp Immunol 1996; **105**:544–550

Morphologically and functionally intact dendritic cells can be derived from the peripheral blood of aged individuals

M. M. STEGER, C. MACZEK & B. GRUBECK-LOEBENSTEIN *Institute of Biomedical Ageing Research of the Austrian Academy of Sciences, Innsbruck, Austria*

(Accepted for publication 18 May 1996)

SUMMARY

Dendritic cells are antigen-presenting cells (APC), which are crucial for the initiation of an immune response. In spite of the well known decline of immune function in old age, no information is yet available on whether dendritic cells are also affected by the ageing process. It was therefore the aim of this study to compare peripheral blood dendritic cells (DC) from old and young healthy individuals. Using granulocyte-macrophage colony-stimulating factor (GM-CSF) and IL-4, DC were propagated from peripheral blood mononuclear cells (PBMC). The obtained cell populations had a typical dendritic morphology and expressed HLA class I and class II, CD23, CD32, CD40, CD44 and CD54, but not CD3 and CD19. Larger numbers of DC were obtained from old individuals than from young ones in spite of a similar expression pattern of surface molecules. DC from aged persons also survived better under *in vitro* culture conditions. When tested for their antigen-presenting capacity, DC from young and old individuals were equally effective in inducing the proliferation of tetanus toxoid-specific T cell clones after antigenic stimulation. Peripheral blood DC from aged individuals may thus still function as powerful APC. They may represent useful tools for immunotherapy in the aged.

Keywords dendritic cells T lymphocytes ageing SENIEUR protocol antigen presentation tetanus toxoid

INTRODUCTION

Dendritic cells (DC), which were first described by Steinman & Cohn in 1973 [1], are a population of widely distributed leucocytes that play a key role in the immune system [2,3]. They are highly specialized in antigen presentation, are the principal activators of resting T cells, and represent a major source of immunogenic epitopes after administration of antigen [2,3]. DC provide T cells with all necessary signals required for activation and proliferation. These signals are generated by the interaction of complexes of MHC molecules and antigenic peptides with the T cell receptor [4] and by the engagement of costimulatory molecules such as the binding of CD54 to CD11/CD18 [5] and of CD80/86 to CD28 and CTLA4 on the T cell surface [6]. Precursors of DC are localized in the bone marrow, from where they emigrate into the peripheral blood [7]. Immature DC may then spread into tissues like epidermis, heart, lung, liver, gut, thymus, spleen and lymph nodes [2,7]. From these organs mature DC emigrate via the lymphoid system after antigen uptake and processing [7].

Correspondence: Dr B. Grubeck-Loebenstein, Institute for Biomedical Ageing Research of the Austrian Academy of Sciences, Rennweg 10, A-6020 Innsbruck, Austria.

DC can be isolated from various sources using sophisticated protocols [8,9]. Due to the low cell numbers available after purification functional studies are, however, difficult to perform. Only recently it has become possible to obtain larger numbers of DC from the peripheral blood [10,11]. This is being done by stimulation with IL-4 and granulocyte-macrophage colony-stimulating factor (GM-CSF). The resulting cells have the phenotypic and functional characteristics of immature DC. They have a typical dendritic morphology, express high levels of the characteristic surface molecules, are highly stimulatory in mixed leucocyte reactions (MLR) and are capable of presenting soluble antigens to specific T cell clones [10,11].

The novel methodological approach to generate lines of functioning DC opened new areas of research. Detailed studies on the function of DC from various physiological and pathological sources can now be performed and defects in antigen presentation elucidated [12,13]. One condition, in which the functional capacity of antigen-presenting cells (APC) is still a matter of debate, is ageing. In spite of a vast literature on the decline of T cell function in old age [14–16], relatively few and controversial studies have been published on APC. Peritoneal macrophages from aged mice have been reported by some groups to have a reduced antigen-presenting capacity [17,18], but were described as unimpaired by

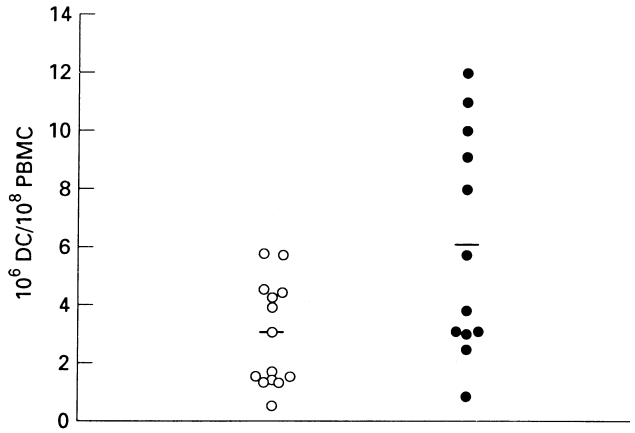

Fig. 1. Yield of dendritic cells (DC) generated from the peripheral blood of young (○) and old individuals (●). Peripheral blood mononuclear cells (PBMC) were incubated for 120 min at 37°C. Non-adherent cells were then removed and the adherent population stimulated with IL-4 (1000 U/ml) and granulocyte-macrophage colony-stimulating factor (GM-CSF; 800 U/ml). After 1 week the cells were removed from the wells, counted and the yield of DC ($10^6/10^8$ PBMC) was assessed. Mean values were 6×10^6 DC in the old and $2{\cdot}9 \times 10^6$ DC in the young group; old *versus* young: $P < 0{\cdot}05$.

others [19,20]. A decreased density and functional activity of Langerhans cells has been observed in the aged mouse skin [21]. In the human, peripheral blood monocytes from aged individuals have been investigated. Their function has mainly been reported to be normal [22,23], but a reduced activity has also been suggested [24,25].

No information is yet available on DC function in old age. It was therefore the aim of the present study to analyse the morphology, phenotype and antigen-presenting capacity of DC lines generated from the peripheral blood of old and young individuals.

MATERIALS AND METHODS

Reagents, monoclonal antibodies and sera

IL-4, GM-CSF and IL-2 were kindly provided by Sandoz (Vienna, Austria), and tetanus toxoid (TT) was a gift from the Schweizer Serum- & Impfinstitut (Bern, Switzerland).

Mouse MoAbs directed against the following cell surface determinants were used: MHC class I (HLA-A, -B, -C; SeraLab, Crawley Down, UK; FITC-conjugated), MHC class II (HLA-DR; FITC-conjugated), CD14 and CD19 (Dako A/S, Glostrup,

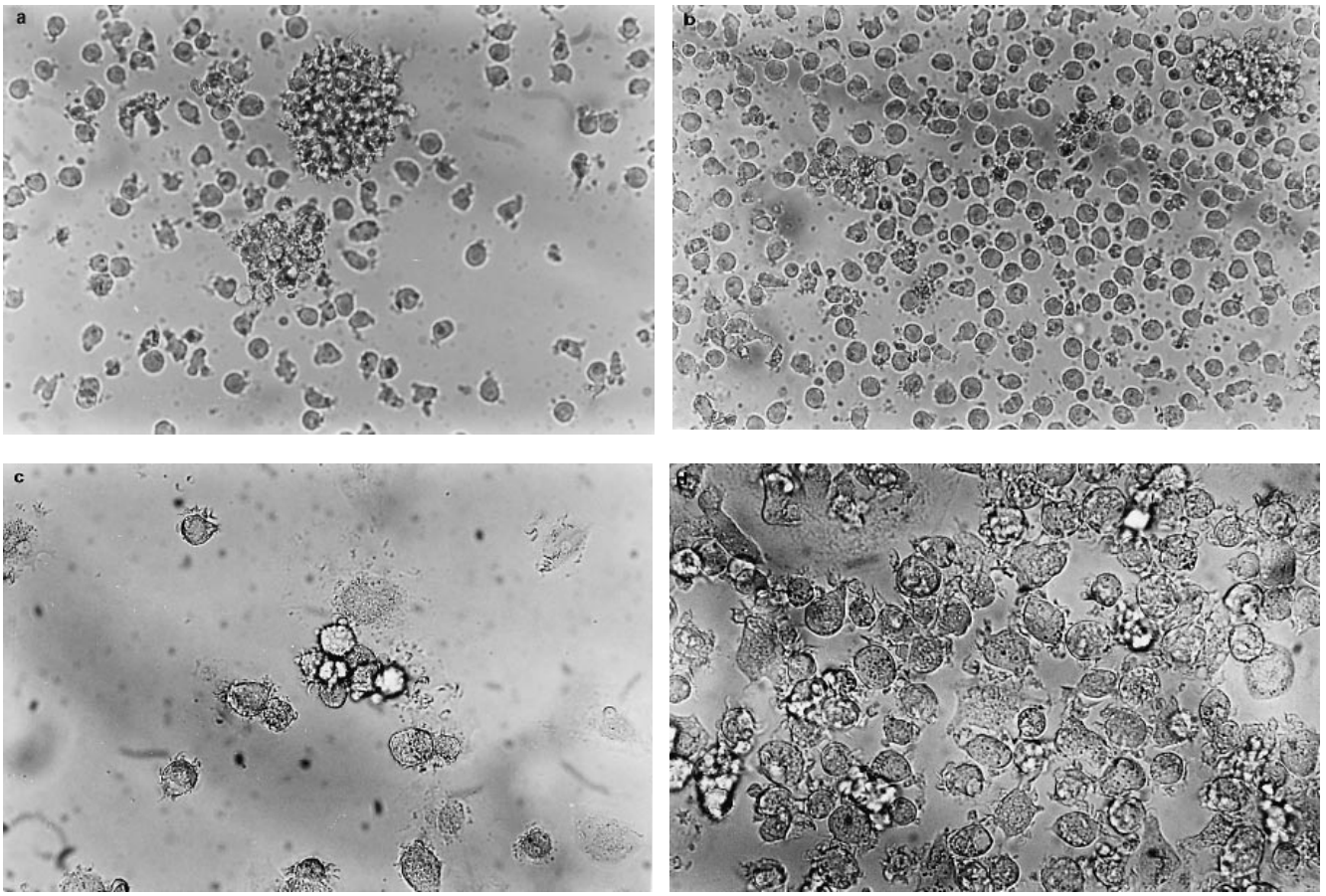

Fig. 2. Morphology of dendritic cells (DC) from old and young individuals. DC were generated from the peripheral blood as described in the legend to Fig. 1. (a,b) The morphology of DC after 1 week of culture (×100 magnification; Labovert FS inverted microscope, Leitz). (c,d) The morphological appearance of the cells after 3 weeks of continuous culture in IL-4 (300 U/ml) and granulocyte-macrophage colony-stimulating factor (GM-CSF; 800 U/ml; ×200 magnification; Labovert FS inverted microscope, Leitz). Two representative cultures, one from a young (a,c) and one from an old individual (b,d) are shown.

Denmark), CD23, CD44, CD54, and CD32 (Monosan, Uden, The Netherlands; FITC- or PE-conjugated), CD40 (Pharmingen, San Diego, CA) and CD3 (An der Grub, Vienna, Austria; PE-conjugated). FITC-conjugated rabbit anti-mouse IgG-IgM was used as second layer (An der Grub).

AB^+ human serum was provided by the Blood Transfusion Unit (University of Innsbruck, Austria), fetal calf serum (FCS) was purchased from SEBAK GmbH (Stuben, Austria).

Preparation of peripheral blood mononuclear cells

Peripheral blood mononuclear cells (PBMC) were obtained from old (>65 years; $n = 12$) and young (<30 years; $n = 14$) healthy blood donors selected according to the SENIEUR protocol of the European Community's Concerted Action Programme on Ageing [26,27]. Cells were purified from heparinized blood by Ficoll–Paque (Pharmacia, Uppsala, Sweden) density centrifugation and washed twice in RPMI 1640 (GIBCO, Grand Island, NY).

Preparation of DC

DC were prepared according to a published method [11]. In brief, PBMC were resuspended in RPMI 1640 containing 10% FCS, 1% antibiotics (P/S; penicillin 10 000 U/ml and streptomycin 10 000 μg/ml; GIBCO) and were allowed to adhere to six-well plates (Falcon; Becton Dickinson, Heidelberg, Germany). After 2 h at 37°C, non-adherent cells were removed and adherent cells cultured at 10^6 cells/ml in RPMI, 10% FCS supplemented with 800 U/ml GM-CSF and 1000 U/ml IL-4. Cells were then fed every other day with fresh culture medium containing 800 U GM-CSF and 300 U IL-4 per ml. After 1 week in culture the cells, which were at that time point mostly non-adherent, were removed from the plate and washed twice in RPMI. They were then counted and analysed. Cell yields were expressed as the numbers of DC obtained from 10^8 PBMC.

Immunofluorescence staining and FACScan analysis

Cells were transferred into round-bottomed tubes (10^5 cells/tube; Becton Dickinson, Mountain View, CA) and washed at 4°C in PBS containing 0·1% FCS. Antibodies were added and the cells left to incubate at 4°C. After 40 min the cells were washed twice in PBS. When antibodies were unconjugated, the first incubation step was followed by a 40-min incubation with a FITC-conjugated anti-mouse IgG-IgM antibody and another washing step. Cells stained with irrelevant antibodies of matching isotypes or with second layer only were used as controls. Analysis was performed on a Becton Dickinson FACScan. Five thousand scatter-gated cells were analysed in each sample. The frequency and fluorescence profiles of the cells were determined with logarithmic signal amplifiers.

Preparation and maintenance of TT-specific T cell lines

Mononuclear cells from two old and from two young persons were stimulated with TT (1 μg/ml) and kept in bulk culture in RPMI 1640 containing 10% human serum for 1 week. Proliferating T cell clusters were then picked under microscopic control, removed into the wells of a 96-well plate (one cluster/well) and restimulated with autologous PBMC (10^5 cells/well, 35 Gy), TT (1 μg/ml) and IL-2 (20 ng/ml). Oligoclonal lines were thus established. After appropriate growth they were transferred to 24-well plates, where they were restimulated with TT in combination with autologous irradiated PBMC (10^6/culture) at weekly intervals. IL-2 was added twice weekly (20 ng/ml), once on day 2 and once on day 4 after the addition of antigen. Phenotypic analysis revealed that the lines were $CD4^+$. Aliquots of the lines were frozen down and kept in liquid nitrogen until analysis.

Proliferation assays

T cells were cultured at 5×10^4 cells/well in flat-bottomed plates in RPMI, 10% human serum. Triplicate cultures were performed in each experiment. T cell proliferation was measured in response to IL-2 (20 ng/ml) as well as in response to TT presented by irradiated autologous PBMC (5×10^4 cells/well) or by irradiated autologous DC (10^4 cells/well). The numbers of PBMC and DC per culture were chosen following a published protocol [11]. Pilot experiments additionally demonstrated that DC numbers could be reduced to 10^3/well without significant changes in their effect. TT had a maximal stimulatory effect at concentrations between 0·1 and 10 μg|ml. It was routinely used at a concentration of 1 μg/ml. T cell proliferation in response to stimulation with autologous irradiated PBMC or DC in the absence of antigen was considered as background. Allogeneic irradiated PBMC and DC were used in control experiments. After a 48-h incubation ^{3}H-thymidine (1 μCi/well) was added. Cultures were then left at 37°C for another 8 h, after which they were harvested and ^{3}H-thymidine incorporation was measured as described [28].

Statistical analysis

Student's *t*-tests were used for statistical evaluation.

RESULTS

DC can be generated from the peripheral blood of old and young individuals

Following a recently established method, in which GM-CSF and IL-4 were used as DC growth stimuli, DC were generated and propagated from the peripheral blood of old and young individuals. Interestingly, the yield of DC was higher when DC were propagated from PBMC of old individuals than when PBMC from young persons were used as a starting population (Fig. 1). Whereas $2{\cdot}9 \pm 0{\cdot}5 \times 10^6$ DC were generated from 10^8 PBMC in young donors, $6 \pm 1{\cdot}1 \times 10^6$ DC could be obtained in the aged donor group ($P < 0{\cdot}05$). The morphological appearence of cultures from young and old individuals was also different (Fig. 2). Whereas in cultures from young donors DC mainly occurred in clusters which were surrounded by a few scattered cells (Fig. 2a), there were fewer clusters and more scattered cells in cultures from aged individuals (Fig. 2b). DC from old individuals also survived longer in culture. After the second week in culture a gradual decrease in growth followed by cellular degradation was mostly observed in DC from young individuals in spite of continuous stimulation with GM-CSF and IL-4 (Fig. 2c). At the same time no such decline was found in many of the cultures from old individuals, in which DC were still proliferating after 3 weeks (Fig. 2d).

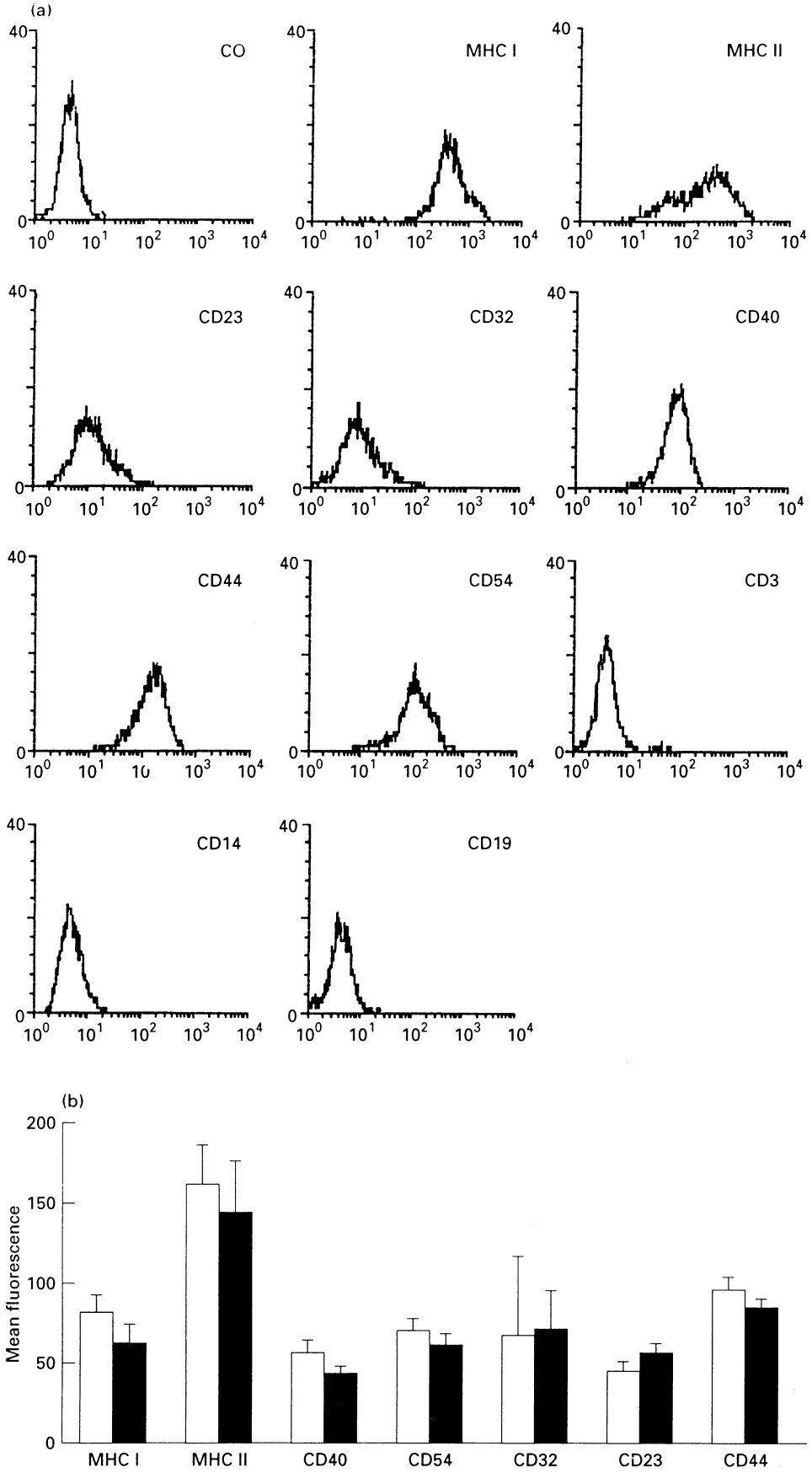

Fig. 3. (See next page for caption.)

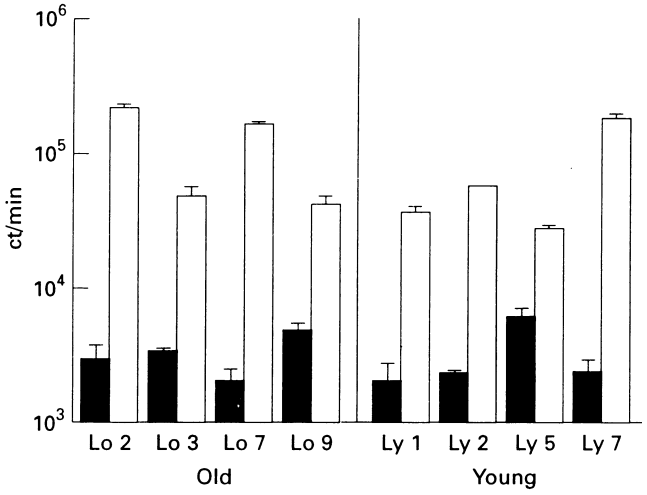

Fig. 4. Dendritic cells (DC) from the peripheral blood of aged individuals have an unimpaired capacity to present antigen to tetanus toxoid (TT)-specific T cell lines. Proliferation (^{3}H-thymidine incorporation) of TT-specific T cell lines (5×10^4 cells/well) in response to stimulation with TT (1 μg/ml) presented by irradiated (30 Gy) autologous peripheral blood mononuclear cells (PBMC; 5×10^4/well) or by irradiated (30 Gy) autologous DC (1×10^4/well). The four T cell lines shown in the left part of the graph derived from two old healthy individuals, the T cell lines on the right hand side were established from two young donors. Results are expressed as mean ct/min ± s.e.m. for triplicate cutures. Proliferation of T cells in response to irradiated antigen-presenting cells (APC) in the absence of antigen was considered as background proliferation and was always less than one tenth of the proliferation in response to antigenic stimulation. No proliferation was noted when TT was added in combination with irradiated allogeneic PBMC or DC. The figure depicts one of four identical experiments. ■, PBMC; □, DC.

DC from old and young individuals have a similar surface marker profile

After 1 week of culture DC from old and young individuals were analysed for their surface expression of MHC class I, MHC class II, CD40, CD54, CD32, CD23, and CD44 (Fig. 3a). In some of the lines from each group CD23 was only present on a subpopulation of DC. All other molecules were expressed by all cells of a population. The intensity of the respective stainings was similar in DC populations derived from young and old individuals (Fig. 3b).

DC from old individuals have an unimpaired capacity to present antigen

To analyse whether DC from aged persons had an intact capacity to present antigen, irradiated DC from two young and two old individuals were pulsed with TT and co-cultured with autologous TT-specific T cell lines. The proliferation of the T cells was then analysed. For comparison, T cell proliferation was also studied when antigen was presented by irradiated autologous PBMC. DC from young and old individuals worked equally well as APC (Fig. 4). In both groups application of TT in combination with DC induced a markedly higher proliferation of T cells than when antigen was presented by PBMC. No T cell response was noted when PBMC or DC were added without antigen or when TT was used in combination with allogeneic DC.

DISCUSSION

Our results demonstrate that DC can be established from the peripheral blood of healthy aged individuals. These cells have a similar surface marker profile to corresponding cells from young persons and have an intact capacity to present antigen. DC have a much better antigen-presenting capacity than PBMC (Fig. 4). This latter feature may be of particular importance in old age, in which T cell responsiveness is diminished [14–16] and clonal elimination of T lymphocytes may take place earlier, after a lower number of population doublings [29–31]. The well known age-related decline of immune function [14] may thus be mitigated by DC, which in the course of immune responses may increase T cell proliferation and thus rescue cells otherwise destined to perish [32]. This may be of relevance for the design of vaccines, which could be more effective when specifically targeted to DC, as recently suggested [7,33].

A requirement for vaccine uptake by DC is a sufficient cell density at the site of injection. Langerhans cell numbers seem to be decreased in the skin of aged mice [21], and humans [34]. The reason for this impairment is still unknown, but it seems possible that the migration of DC from the blood stream to peripheral organs is affected by the ageing process. As the expression of surface molecules is unimpaired in DC from aged individuals (Fig. 3), it is unlikely that the DC themselves do not meet the necessary requirements for migration. It is still possible that due to changes of the endothelium [35] and alterations of extracellular matrix proteins [36] the mobility of DC is reduced. A decreased emigration of DC into the periphery and increased numbers of immature DC in the blood stream would be the consequence. This concept is supported by our finding that the yield of immature DC from the blood was considerably higher in old than in young individuals (Fig. 1). DC from aged persons also had a different morphology and improved survival qualities *in vitro*. This suggests that a DC population of high quality, which could migrate quickly in the young, may be retained for longer periods in the circulation in aged individuals. Under which conditions these cells could finally leave the blood stream will be a topic of future investigations. It seems still possible that DC may be functioning while circulating in the blood stream. They may, for

Fig. 3. (See previous page.) Immunofluorescence staining and analysis of dendritic cell (DC) lines grown from the peripheral blood with IL-4 and granulocyte-macrophage colony-stimulating factor (GM-CSF). Cells were stained with different MoAbs as described in Materials and Methods. (a) One characteristic experiment, in which cells from a young individual were used. The curves represent the phenotype of the population. A control experiment in which cells were stained with second layer only is shown in the upper panel. It is notable that the population did not express CD3, CD14 and CD19, but MHC class I, MHC class II, CD23, CD32, CD40, CD44, and CD54. (b) Comparison of the different stainings in DC from young (□) and old (■) individuals. The bars represent the mean channel fluorescence. Results are expressed as means ± s.e.m. ($n = 10$ in the young and $n = 9$ in the old group).

instance, meet and incorporate certain antigens such as bacterial toxins [37]. Incorporation of antigen could then lead to DC maturation. Extravasation and migration to lymphoid tissues might be the consequence [7].

In conclusion, our results for the first time demonstrate that intact DC which may function as potent APC frequently occur in the blood stream of aged individuals. As these cells could represent useful tools for immunotherapy [7,38], efforts should be made to acquire further knowledge of the maturation and migration of DC in the aged.

ACKNOWLEDGMENTS

We are grateful to G. Wick for his continuous encouragement and support and to N. Romani for technical advice. We would also like to thank the companies referred to in the text for their generous gift of reagents. This study was supported by a grant from the Jubiläumsfond of the Austrian National Bank No. 5568 and the state of Tyrol, Austria.

REFERENCES

1 Steinman RM, Cohn ZA. Identification of a novel cell type in peripheral lymphoid ogans of mice. J Exp Med 1973 **137**:1142–8.
2 Steinman RM. The dendritic cell system and its role in immunogenicity. Annu Rev Immunol 1991; **9**:271–96.
3 Romani N, Schuler G. The immunologic properties of epidermal Langerhans cells as a part of the dendritic cell system. Springer Semin Immunopathol 1992; **13**:265–79.
4 Davis M, Bjorkman PJ. T cell antigen receptor genes and T cell recognition. Nature 1988; **334**:395–402.
5 van Seventer GA, Shimizu Y, Horgan KJ, Shaw S. The LFA-1 ligand ICAM-1 provides an important costimulatory signal for T cell receptor-mediated activation of resting T cells. J Immunol 1990; **144**:4579–86.
6 Linsey PS. Distinct roles for CD28 and cytotoxic T lymphocyte associated meolcule-4 receptors during T cell activation. J Exp Med 1995; **182**:289–92.
7 Caux C, Liu YJ, Banchereau J. Recent advances in the study of dendritic cells and follicular dendritic cells. Immunol Today 1995; **16**:2–4.
8 Romani N, Lenz A, Gassl H *et al.* Cultured human Langerhans cells resemble lymphoid dendritic cells in phenotype and function. J Invest Dermatol 1989; **93**:600–9.
9 Pavli P, Hume DA, Van de Pol E, Poe WF. Dendritic cells, the major antigen-presenting cells of the human colonic lamina propria. Immunology 1993; **78**:132–41.
10 Romani N, Gruner S, Brang D *et al.* Proliferation of dendritic cell progenitors in human blood. J Exp Med 1994; **180**:83–93.
11 Sallusto F, Lanzavecchia A. Efficient presentation of soluble antigen by cultured human dendritic cells is maintained by granulocyte/macrophage colony-stimulating factor plus interleukin-4 and down-regulated by tumor necrosis factor-α. J Exp Med 1994; **179**:1109–18.
12 Woods GM, Chilcott AR, Grzegorczck AD, Field CM, Ragg SJ, Muller HK. Analysis of dendritic cells during carcinogen induced immunosuppression. 9th International Congress of Immunology, San Francisco 1995; 2428 (Abstr.).
13 Drexhage HA, Hooijkaas H, Jansen A. Evidence for a defective function of dendritic cells in endocrine autoimmne disease. A factor of importance in the disturbed tolerance towards endocrine cell antigens? 9th International Congress of Immunology, San Francisco 1995; 3718 (Abstr.).
14 Miller RA. Aging and the immune response. In: Schneider L, Rowe J, eds. Handbook of the biology of aging. San Diego: Academic Press, 1990:157–81.
15 Globerson A. T lymphocytes and aging. Int Arch Allergy Immunol 1995; **107**:491–7.
16 Pawelec G. Molecular and cell biological studies of aging and their application to considerations of T lymphocyte immunosenescence. Mech Aging Dev 1995; **79**:1–32.
17 Vetvicka V, Tlaskalova-Hogenova H, Pospisil M. Impaired antigen presenting function of macrophages from aged mice. Immunol Invest 1985; **14**:105–14.
18 De la Fuente M. Changes in the macrophage function with aging. Comp Biochem Physiol A 1985; **81**:935–8.
19 Perkins EH, Massucci JM, Glover PL. Antigen presentation by peritoneal macrophages from young adult and old mice. Cell Immunol 1982; **70**:1–10.
20 Komatsubara S, Cinader B, Muramatsu S. Functional competence of dendritic cells of ageing C57/BL/6 mice. Scand J Immunol 1986; **24:**517–25.
21 Sprecher E, Becker Y, Kraal G, Hall E, Harrison D, Shultz LD. Effect of aging on epidermal DC populations in C57BL/6J mice. J Invest Dermatol 1990; **94**:247–53.
22 Schwab R, Hausman PB, Rinnooy-Kan E, Weksler ME. Immunological studies of aging X. Impaired T lymphocytes and normal monocyte response from elderly humans to the mitogenic antibodies OKT3 and Leu4. Immunology 1985; **55**:677–84.
23 Fagiolo U, Hausman PB, Rinnooy-Kan E, Weksler ME. Increased cytokine production in mononuclear cells of healthy elderly people. Eur J Immunol 1993; **23**:2375–8.
24 Rich EA, Mincek MA, Armitage KB, Duffy EG, Owen DC, Fayen JD, Hom DL, Ellner JJ. Accessory function and properties of monocytes from healthy elderly humans for T lymphocyte responses to mitogen and antigen. Gerontology 1993; **39**:93–108.
25 Beckman I, Dimopoulos K, Xaioning X, Bradley J, Henschke P, Ahern M. T cell activation in the elderly: evidence for specific deficiencies in T cell/accessory cell interactions. Mech Ageing Dev 1990; **51**:265–76.
26 Ligthart GJ, Corberand JX, Fournier C, Galanaud P, Hijmans W, Kennes B, Müller-Hermelink HK, Steinmann GG. Admission criteria for immunogerontological studies in man: the Senieur protocol. Mech Ageing Dev 1984; **28**:47–55.
27 Reibenegger G, Huber LA, Jürgens G, Schönitzer D, Werner ER, Wachter H, Wick G, Traill KN. Approach to define 'normal aging' in man. Immune function, serum lipids, lipoproteins and neopterin levels. Mech Ageing Dev 1988; **46**:67–82.
28 Grubeck-Loebenstein B, Trieb K, Sztankay A, Holter W, Anderl H, Wick G. Retrobulbar T cells from patients with Graves' ophthalmopathy are $CD8^+$ and specifically recognize autologous fibroblasts. J Clin Invest 1994; **93**:2738–43.
29 Grubeck-Loebenstein B, Lechner H, Trieb K. Long-term *in vitro* growth of human T cell clones: can postmitotic 'senescent' cell populations be defined? Int Arch Allergy Immunol 1994; **104**: 232–9.
30 Walford RL, Jawaid SQ, Naeim F. Evidence for *in vitro* senescence of T lymphocytes cultured from normal human peripheral blood. Age 1981; **4**:67–70.
31 McCarron M, Osborne Y, Story CJ, Dempsey JL, Turner DR, Morley AA. Effect of age on lymphocyte proliferation. Mech Ageing Dev 1987; **41**:211–8.
32 Lechner H, Amort M, Steger MM, Maczek C, Grubeck-Loebenstein B. Regulation of CD95 (APO-1) expression and the induction of apoptosis in human T cells: changes in old age. Int Arch Allergy Immunol 1996; **110**:238–43.
33 Baier G, Baier-Bitterlich G, Looney DJ, Altman A. Immunogenic targeting of recombinant peptide vaccines to human antigen-presenting cells by chimeric anti-HLA-DR and anti-sIgD antibody Fab fragments *in vitro*. J Virol 1995; **69**:2357–65.
34 Gilchrest BA, Murphy GF, Soter NA. Effect of chronological aging on

Langerhans cells in the human epidermis. J Invest Dermatol 1982; **79**:85–88.
35 Cooper LT, Cooke JP, Dzau VJ. The vasculopathy of aging. J Gerontol 1994; **49:**B191–6.
36 Kefalides NA, Alper R. Aging and connective tissues. In: Brocklehurst JC, Tallis RC, Fillit H, eds. Textbook of geriatric medicine and gerontology. London: Churchill Livingstone, 1993:61–70.
37 Gotschlich EC. Immunity to extracellular bacteria. In: Paul WE, ed. Fundamental immunology. New York: Raven Press, 1993: 1287–308.
38 Stingl G, Bergstresser PR. Dendritic cells: a major story unfolds. Immunol Today 1995; **16**:330–3.