PERITONEAL DENDRITIC CELLS AS A POSSIBLE TARGET FOR IMMUNOTHERAPY

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Dendritic cells (DC) are the most important antigen–presenting cells (APC) (Bancherau et al., 1998). They are located in many tissues and organs, including peritoneal cavity. DC represent a minor subset of peritoneal cells (Betjes et al., 1993a). These cells together with macrophages may play a role in presentation of antigens to T cells that enter the peritoneal cavity. However, DC are the only cells capable of triggering proliferation of naive T cells (Betjes et al., 1993b; Fong et al., 2000). During the last decade, based on the efforts of scientists worldwide, different protocols for isolation, cultivation and maturation of DC in vitro from their precursors (bone marrow, umbilical $CD34^+$ cells, monocytes) were established. In addition, a significant part of adherent macrophages could be converted to non–adherent immunogeneic DC under specific in vitro culture conditions (Banchereau et al., 1998; Obermaier et al. 2003). Up to now tha data about the phenotype and function of peritoneal DC and their differentiation potential from peritoneal macrophages in vitro are very scarce (Kubicka et al., 1996; Puing–Kroger et al., 2003). In this work we studied phenotypic and functional characteristics of DC generated in vitro from peritoneal macrophages. Peritoneal macrophages were isolated from dialysis effluents of patients in the early stage of continious ambulatory peritoneal dialysis (CAPD). These cells could be a possible target for immunotherapy in CAPD patients with recurent episodes of peritonitis or patients with different forms of carcinomas.

MATERIALS AND METHODS

Six patients in the eraly stage of CAPD were included in this study. Peritoneal cells collected from dialysis effluents and peripheral blood mononuclear cells, isolated using Lymphoprep gradient, were cultivated for 1h in 6–well plates at 37°C. Non–adherent cells were removed, whereas adherent cells (macrophages and monocytes, respectively) were cultivated for 7 days with IL–4 (5 ng/ml) and GM–CSF (100 ng / ml) in RPMI medium +10% fetal calf serum (FCS) as we previously described (Čolić et al., 2003). Maturation of peritoneal macrophage–derived DC (PMDC) and monocyte– derived DC (MDC) was induced by addition of lypopolysaccharide (LPS) (1 μg/ml) and subsequent cultivation of these cells for 2 days.

Phenotypic characteristics of DC were assessed by single and double immunofluorescence and flow cytometry (EPICS XL–MCL) using monoclonal antibodies (mAbs) to CD14 and CD83 directly conjugated with fluorescein isothiocyanate (FITC) as well as CD1a conjugated with phycoerythrin (PE). Endocytosis of PMDC and MDC was tested using a dextran–FITC uptake assay (Čolić et al., 2003).

Alloreactivity was tested using classical mixed leukocyte reaction (MLR). Peripheral blood mononuclear cells (PBMNC), isolated from buffy coats using Lymphoprep gradient, were used as responders. Allogeneic PMDC and MDC were used as stimulators. Cells were cultivated in complete RPMI–1640 medium with 10% FCS in 96–well culture plates for 6 days. Cells were pulsed with 1μCi/well of [3H]–tymidine (Amersham, Bucks, UK) for the last 18h. Radioactivity was counted and expressed as counts per minute (cpm) ±SD of triplicates (Čolić et al., 2003).

RESULTS AND DISCUSSION

DC were generated in vitro by cultivating peritoneal macrophages or monocytes from patients in the early stage of CAPD with GM–CSF and IL–4. After 7 days most adherent cells became non–adherent cells with dendritic morphology. As presented in Fig. 1A MDC down–regulated CD14 and expressed CD1a, a DC marker (Chia–Chun et al., 2000) on about 60% cells. The rest of DC (about 40%) were $CD1a^-CD14^-$. In cultures

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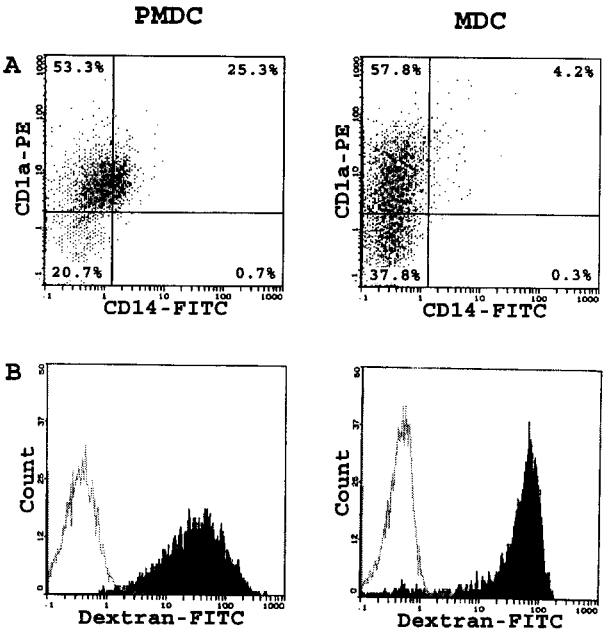

Figure 1. Phenotypic (A) and endocytic (B) characteristics of PMDC and MDC

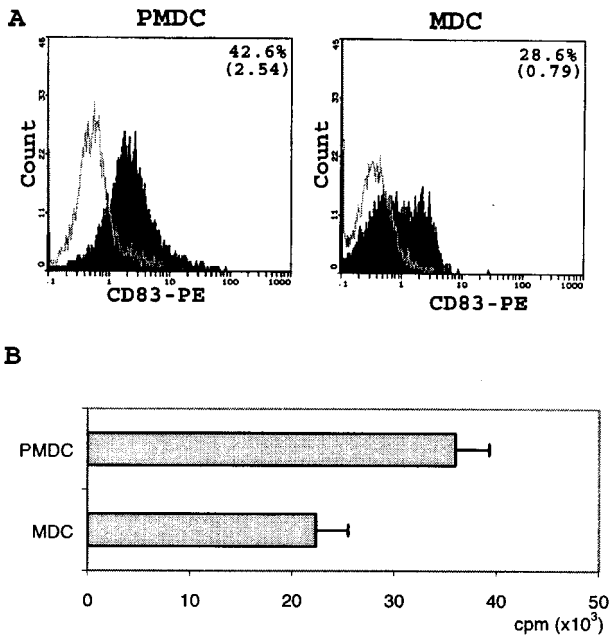

Figure 2. Phenotypic (A) and allostimulatory (B) characteristics of PMDC and MDC matured in the presence of LPS

with peritoneal macrophages three different DC subsets were identified: $CD1a^+CD14^-$ (about 55%); $CD1a^-CD14^-$ (about 20%) and $CD1a^+CD14^+$ (about 25%). Results of Chia–Chun et al., 2000 showed that both $CD1a^+CD14^-$ and $CD1a^-CD14^-$ cells recovered from monocyte cultures stimulated with GM–CSF and IL–4 are DC but these subsets differ in their cytokine production profiles, susceptibilities to transfection and capacities to direct Th cell differentiation. $CD1a^+CD14^+$ cells in our cultures are probably a transitional stage between macrophages and DC.

Fig. 1B shows that PMDC and MDC had comparable endocytic potential. These results suggest that PMDC could probably phagocytose other particles such as biocompatible biospheres containing different delivery components (Newman et al., 1998). In this contest, recent published data that poly (D,L–lactic–co–glycolic acid) (PLGA) nanoparticles were significantly taken up by mature DC (Coeseter et al., 2004) are very promising to perform similar experiments with human PMDC.

Phenotypic characteristics of immature PMDC and MDC were determined by flow cytometry after staining of cells with CD1a–PE and CD14–FITC mAbs. Control cells were stained with irrelevant PE– and FITC–conjugated mAbs. Endocytosis was performed by incubating immature PMDC and MDC with dextran–FITC (1 μg/ml) at 37 C for 1h (black histograms). The analysis of fluorescent cells was performed by flow–cytometry. Control cells were incubated at +4C (white histograms). Results are from one representative experiment.

Immature DC were induced to mature with LPS, a well–know DC maturation marker and a potent stimulator of IL–12 production by DC (Banchereau et al., 1998; Fong et al., 2000; Obermaier et al., 2003). PMDC better respond to LPS than MDC, as judged by higher up–regulation of CD83 expression (Fig. 2A) and higher capability to stimulate proliferation of allogeneic lymphocytes (Fig. 2B). These results, that are the first ones published so far in this research field in human, suggest that DC generated in vitro from peritoneal macropahges are potent APC. It can be expected that PMDC matured in the presence of LPS are able to stimualte Th1 immune response. Our unpublished data showed that LPS–treated PMDC produce high levels of IL–12, a potent Th1 inducing cytokine. It is well known that Th1 immune response is needed for anti–tumor immunity and immunity to intracellular microorganisms (Banchereau et al., 1998; Fong et al., 2000).

The possibility to collect large numbers of PMDC from CAPD and from other patients, to freeze and modify these cells in vitro in different ways, open a new strategy to use them as an alternative DC target for immunotherapy.

Phenotype of mature PMDC and MDC were determined by flow cytometry after staining of the cells with CD83–FITC mAb (black histograms). Control cells were stained with an irrelevant, FITC–conjugated mAb (white histograms). For testing the allostimulatory activity DC ($5x10^3$/well) were cocultured with allogeneic lymphocytes ($2x10^5$ cells/well) in 96–well plates for 6 days. During the last 18h of incubation cultures were pulsed with 3H–thymidine (1μCi/well). Proliferation was measured as count per minute (cpm). Results are given as mean cpm ±SD of triplicates of one representative experiment.

CONCLUSION

In the presence of GM–CSF and IL–4 most adherent peritoneal macrophages from patients in the early stage of CAPD transformed into DC. PMDC were phenotypically different from MDC. In the presence of LPS, PMDC better matured and stimualted proliferation of allogeneic lymphocytes than MDC. These cnaracteristics make them attractive as a target for biocompatible nanoparticulate delivery systems in order to be used for immunotherapy in vivo.

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