Voltage-gated K^+ currents of mouse dendritic cells

Hans-Georg Fischer[a] and Claudia Eder[b,*]

[a]*Institut für Medizinische Mikrobiologie und Virologie, Heinrich-Heine-Universität, Universitätsstr. 1, D-40225 Düsseldorf, Germany*
[b]*Abt. Neurophysiologie, Institut für Physiologie der Charité, Humboldt Universität zu Berlin, Tucholsky Str. 2, D-10117 Berlin, Germany*

Received 29 August 1995

Abstract Dendritic cells (DC) were enriched from murine spleen by exploiting their intermediate density and transient weak adherence. The isolated population contained excellent antigen presenting cells with high surface expression of major histocompatibility complex (MHC) class II determinants thus exhibiting crucial immunofunctional characteristics of DC. Cells of typical dendritic shape were electrophysiologically analysed using the whole cell configuration of the patch clamp technique. All 26 cells expressed only outward K^+ currents comparable to those detected in cytokine-activated microglia. Co-purified splenic macrophages, in contrast, displayed an inward rectifying K^+ current.

Key words: Dendritic cell; MHC class II; Antigen presentation; Patch clamp; Potassium current

1. Introduction

Dendritic cells (DC) are a population of specialized immune inducer cells which stem from myeloid bone marrow precursors and are found in traces in both non-lymphoid and lymphoid tissues. These cells were first identified due to their veiled or dendritic morphology [1] combined with an extraordinary high membrane expression of MHC class II molecules. Dendritic cells are motile, they capture antigens and initiate directed T cell responses due to their constitutive ability for MHC class II-restricted antigen presentation [2]. Consistent with this immunofunctional profile, DC also show strong surface expression of other ligands for T cells such as CD1, ICAM-1 or LFA-3 [2]. In addition, DC share phenotypic markers with cells of the monocyte/macrophage lineage [3], however a relationship to these cells is contentious.

Macrophages, the cell type most closely related to DC, are electrophysiologically well characterized [4]. A voltage-gated inward rectifying K^+ current (I_{IR}) has been detected on all macrophage populations examined [5,6]. Additionally, an outward K^+ current (I_K) was shown to be exhibited by blood-derived and brain macrophages following activation in vivo or in vitro [7,8]. A cell type-specific [6] versus a functional state-dependent [8,9] pattern of I_{IR}/I_K expression has been postulated. Dendritic cells, which so far are not electrophysiologically characterized, were studied now with regard to both explanations. In this context, we describe here the K^+ current pattern recorded in DC from murine spleen which seems comparable to that of cytokine-activated brain macrophages [9].

2. Materials and methods

2.1. Mice

(C57BL/10 × C3H/HeJ)F_1 hybrids were bred from parental inbred strains originally supplied by the Zentralinstitut für Versuchstierforschung (Hannover, Germany). Two to three month old animals of either sex were used.

2.2. Preparation of splenic DC

The medium used during all steps of cell purification was IMDM (Gibco) supplemented with 2 mM L-glutamine, 5×10^{-5} M β-mercaptoethanol and 5% heat-inactivated FCS (Biochrom, Berlin, Germany). A single cell suspension of splenocytes was prepared from triturated organ halves. Red cells were lysed with ammonium chloride. The leukocytes were washed and fractionated over a 1.04/1.07 Percoll gradient analogous to [10]. Interface cells were collected, washed again and depleted of non-adherent granulocytes after 2 h incubation (2×10^7 cells/10 ml) at 37°C in Petri dishes (Nunc, Wiesbaden, Germany). Medium was then renewed for a subsequent overnight incubation during which loosely adherent cells detached. These cells were harvested for the staining procedure or for patch clamp analyses. Aliquots of 3×10^4 cells/ml were seeded onto serum-coated glass coverslips in 24-well plates (Costar). The medium was supplemented with additional 5 ng/ml of recombinant murine GM-CSF (Behringwerke, Marburg, Germany) to maintain the viability of DC. In order to circumvent their progressive loss of T cell stimulatory activity the cells for antigen presentation assays were harvested after the first adherence stage using a teflon cell scraper (Falcon). This enriched population was γ-irradiated and then tested.

2.3. Immunofluorescence staining of surface MHC class II

The result of DC concentration was checked via indirect immunofluorescence staining of I-A^k_β determinants with mAb 10–2.16 [11]. Cells on glass coverslips were fixed using acid methanol. Samples were then washed and incubated with mouse IgG2a mAb 10–2.16 purified from hybridoma (ATCC) supernatant and diluted to 0.1 μg/ml in PBS with 10% FCS and 1% goat serum (Dianova, Hamburg, Germany). After washing, a FITC-conjugated $F(ab')_2$ fragment of goat anti-mouse IgG (Dianova), diluted 1:50, served as detection antibody. Samples were examined using a Zeiss photomicroscope III (Zeiss Oberkochen, Germany).

2.4. Testing for antigen presentation

The capability of enriched splenic DC to induce antigen-dependent proliferation of cloned $CD4^+$ T cells was determined using syngeneic 3Tx T_H1 cells [12] which recognize Toxoplasma antigen in the context of A^b_α A^k_β MHC class II molecules. Assay medium was IMDM (Gibco) with glutamine, β-mercaptoethanol and 5% FCS. In flat bottom microtiter plates (Nunc) irradiated and fractionated splenocytes (titrated 3×10^3–4×10^5/200 μl/well) were co-cultured with 3Tx T cells (2×10^4/well) in the presence or absence of antigen (0.3 μg/ml of Toxoplasma lysate) prepared as described [12]. The suboptimal dose of antigen was chosen to reveal differences in the ability for antigen presentation by various splenocyte fractions. After 2 days of incubation the test cultures were pulsed with 0.2 μCi = 7.4 kBq tritiated thymidine for a further 18 h and processed for liquid scintillation counting. Results are expressed as mean cpm ± S.D. of triplicate cultures representing the antigen-induced proliferative T cell response.

*Corresponding author. Fax: (49) (30) 28026669.

Abbreviations: DC, dendritic cells; FCS, fetal calf serum; GM-CSF, granulocyte/macrophage colony-stimulating factor; ICAM, intercellular adhesion molecule; I_{IR}, inward rectifying potassium current; I_K, outward potassium current; I_K', frequency-independent I_K; IMDM, Iscove's modified Dulbecco's medium; LFA, lymphocyte function associated antigen; mAb, monoclonal antibody; MHC, major histocompatibility complex; PBS, phosphate-buffered saline.

SSDI 0014-5793(95)01029-7

2.5 Patch clamp analysis

Membrane currents were measured using the whole-cell configuration of the patch clamp technique [13]. An EPC-9 patch clamp amplifier was interfaced to an Atari computer for pulse application and data recording. Series resistance compensation was routinely used to reduce the effective series resistance by about 70%. Patch electrodes of 4–6 MΩ were fabricated from borosilicate glass (outer diameter 1.5 mm, inner diameter 1.0 mm; Hilgenberg, Malsfeld, Germany). For current recordings the electrodes were filled with a high K^+ solution (in mM): KCl 120, $CaCl_2$ 1, $MgCl_2$ 2, HEPES 10, EGTA 11, D-glucose 20 (pH = 7.3). In some cases KCl was substituted by CsCl. The extracellular solution contained (in mM): NaCl 120, KCl 5.4, $CaCl_2$ 2, $MgCl_2$ 1, HEPES 10, D-glucose 25 (pH = 7.4). All recordings were done at room temperature (20–23°C). Voltage-gated currents were filtered at 3 kHz and stored on computer disk for subsequent analyses. Analyses were performed on Atari computers with the Review program (Instrutech, Mineola, USA). Current recordings were not subtracted for leak currents which were very small compared with the voltage-gated currents.

3. Results and discussion

Dendritic cells were enriched from mouse splenocytes by purifying them through a density gradient followed by different adherence steps. The use of this protocol [10] resulted in about 0.1% cell recovery with the majority of isolated cells displaying the typical veiled shape of DC with spherical somata and sheet-like processes (Fig. 1A). Among the co-purified cells of non-DC morphology, macrophages represented the most common cell type. The identity of veiled cells was controlled via indirect immunofluorescence staining of surface MHC class II determinants. Within the cell population isolated only DC proved to carry high levels of MHC class II products as demonstrated in Fig. 1B, while accompanying macrophages were negative. As a functional criterion which defines DC, the capability for antigen presentation was determined. As can be seen from Fig. 2, in the course of purification procedure highly efficient antigen presenting cells were enriched. After 2 of the 3 purification steps, the number of cells required for half-maximum antigen-directed T cell response was remarkably lower compared to unseparated splenocytes. Therefore, according to phenotypical and immunological properties of the cells obtained, DC had been enriched and were shown to be functionally active.

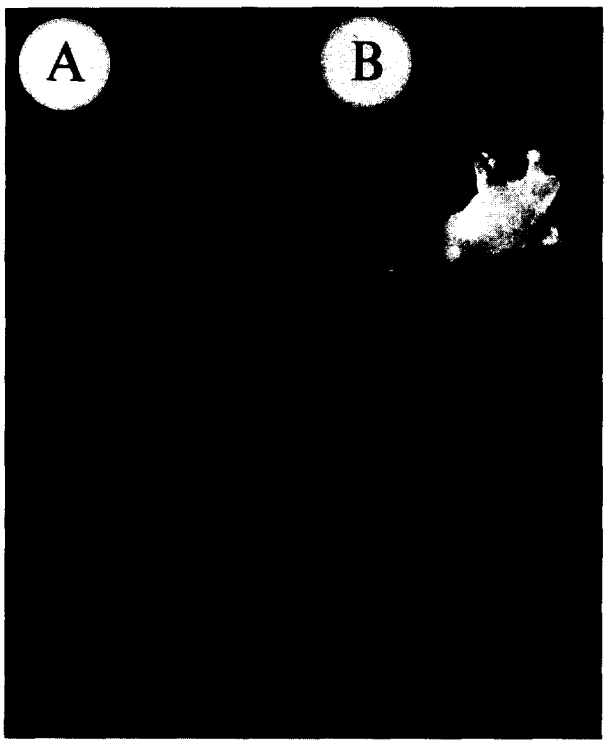

Fig. 1. Morphological and phenotypical characteristics of the DC analysed. Cells were isolated from murine splenocytes by the classical enrichment procedure to obtain DC (see section 2.2.). (A) Phase contrast, bar = 10 μm. (B) Immunofluorescence staining of MHC class II markers.

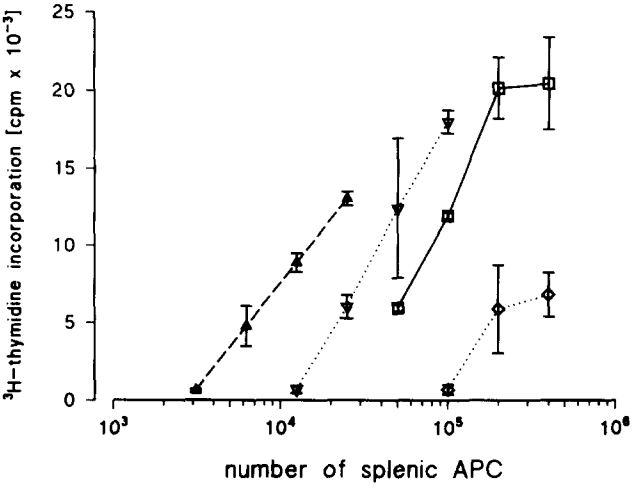

Fig. 2. Antigen presentation function of spleen cell fractions. Spleen cells were separated over Percoll density gradient followed by one adherence step. 3Tx T cells were co-cultured with titrated numbers of either unseparated splenocytes (□) or highly dense cells from the pellet (◇), or interface cells (▽) and adherent cells from the interface (▲), respectively, where DC are enriched. T cell proliferation was challenged in the presence of antigen and was measured by [^{3}H]thymidine incorporation on day 3 of incubation. Control cultures lacking spleen cells or antigen or T cells yielded ≤ 99 cpm, ≤ 529 cpm and ≤ 156 cpm, respectively.

Twenty-six of the isolated DC and 11 concomitant macrophage-like cells were electrophysio-logically analysed using the patch clamp technique. After establishing the whole-cell configuration all cells were clamped to a holding potential of −60 mV. Hyperpolarizing (up to −150 mV) or depolarizing (to +30 mV) voltage commands were applied for 200 ms in 10 mV increments. Dendritic cells displayed an I_K upon depolarization but did not show any K^+ current in response to hyperpolarizing pulses (Fig. 3A). The threshold of activation of I_K was determined at about −40 mV which is consistent to data from the outward K^+ currents of other leukocytes [4]. The corresponding current–voltage curve for the example given in Fig. 3A shows that I_K increased at more depolarizing test potentials (Fig. 3C). Upon removal of K^+ from the intracellular solution I_K was not detectable.

By contrast, an I_{IR} (Fig. 3B) was recorded in every splenic macrophage analyzed. In 6 of 11 cells this current was exhibited together with the I_K. As expected for an inward rectifier, I_{IR} activated at potentials more negative than −80 mV, i.e. negative to the adjusted K^+ equilibrium potential (Fig. 3C). Following substitution of internal K^+ by Cs^+ no I_{IR} was measurable.

Furthermore, dendritic cells were checked for a possible fre-

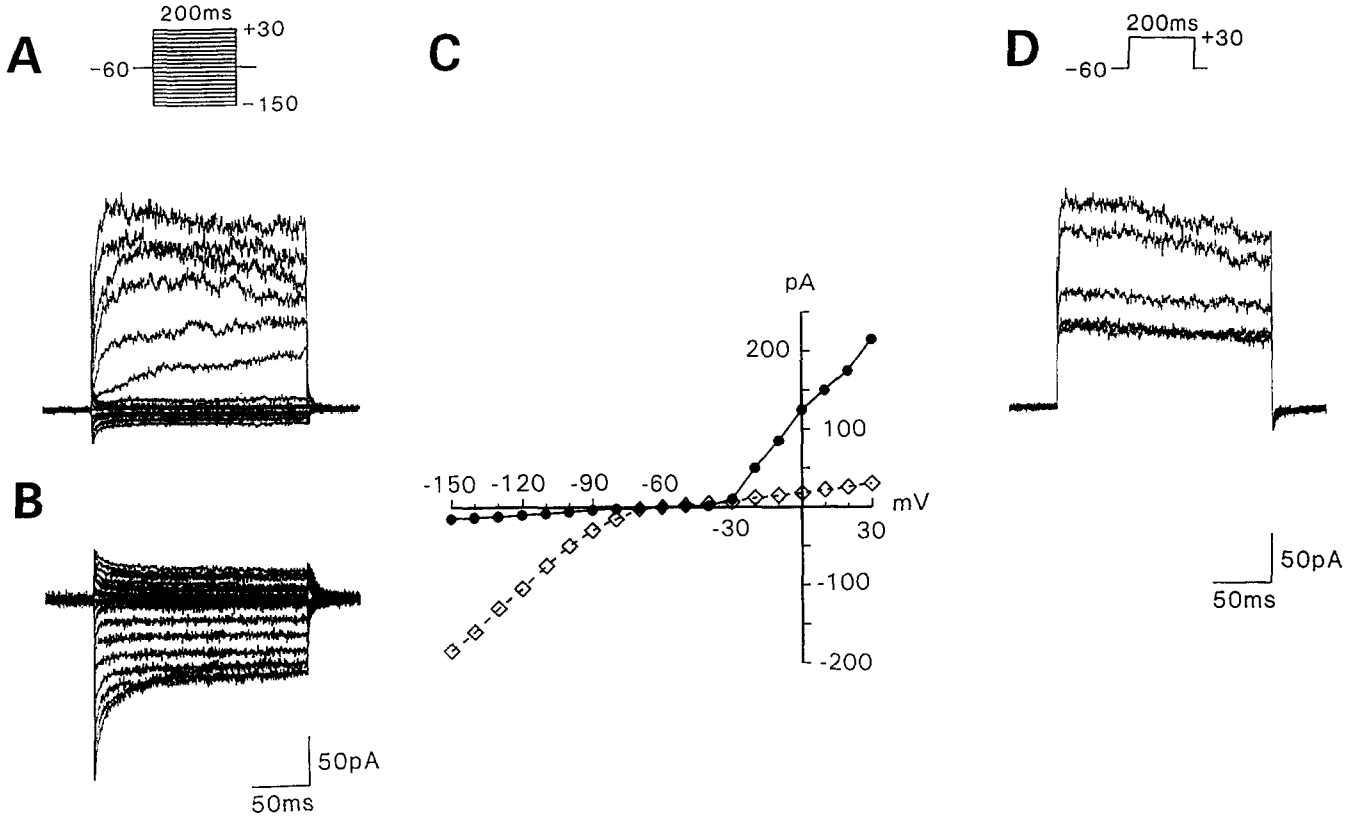

Fig. 3. Potassium currents in splenic DC and macrophages. (A) Dendritic cells exhibit only K^+ outward currents in response to voltage steps from the holding potential (−60 mV) to potentials between −150 and +30 mV. (B) Expression of I_{IR} by a spleen macrophage in response to the same voltage protocol. (C) Corresponding current–voltage curves of the peak currents for the examples shown in A (closed symbols) and B (open symbols). (D) Application of 200 ms pulses to +30 mV at a frequency of 1 Hz unmasked a frequency independent I_K component in DC. The 1st, 2nd and 5th traces are shown and superimposed on each other the 10th, 20th and 30th traces.

quency-independent component of I_K (I_K') which seems to be characteristic for the voltage-gated outward K^+ currents in GM-CSF-activated brain macrophages as well as in T lymphocytes [13, 14]. Repetitive voltage pulses to +30 mV of 200 ms duration were applied with a frequency of 1 Hz. As demonstrated in Fig. 3D the amplitude of I_K rapidly decreased and reached a frequency-independent level at about 40% of the amplitude of the first given pulse. This behaviour of the I_K in DC corresponds to that of its counterpart detected in brain macrophages.

In this report electrophysiological characteristics of DC are demonstrated for the first time and reveal a similarity to other leukocytes [4]. With regard to their exhibition of sole I_K and its activation profile, DC isolated from mouse spleen resemble cultured brain macrophages which were cytokine-activated either by exposure to interferon-γ or intrinsically upon differentiation with GM-CSF [9]. The current pattern observed in DC is unlikely to be a result of GM-CSF application during the last step of cell purification since (i) a great proportion of co-purified macrophages proved not to be influenced and displayed only I_{IR} (see Fig. 3B), and (ii) a 40-fold higher dose of GM-CSF was required to alter the I_K/I_{IR} ratio in cultured brain macrophages [9].

The coincidence of predominant I_K expression and of an elevated immuno-functional state as found in cultured brain and blood macrophages [7,15] can also be noted at DC whose functional activity was demonstrated by their excellent antigen presentation. Whether and how an altered cellular reactivity for changes in their K^+ homeostasis participates in the antigen presentation function of DC, is still unclear. Nevertheless, the detection of I_K may provide an electrophysiological marker for an actually elevated functional state of cells which can be measured at the single cell level.

Acknowledgements: We are grateful to B. Nitzgen for the excellent preparation of cells and for technical assistance and Prof. H. Haas for generously providing us with his electrophysiological laboratory. This work was supported by the Deutsche Forschungsgemeinschaft, projects B11/SFB 194 and C3/SFB 507.

References

[1] Steinman, R.M. and Cohn, Z.A. (1973) J. Exp. Med. 137, 1142–1162.
[2] Steinman, R.M. (1991) Annu. Rev. Immunol. 9, 271–296.
[3] Inaba, K., Inaba, M., Romani, N., Aya, H., Deguchi, M., Ikehara, S., Muramatsu, S. and Steinman, R.M. (1992) J. Exp. Med. 176, 1693–1702.
[4] Gallin, E.K. (1991) Physiol. Rev. 71, 775–811.
[5] McKinney, L.C. and Gallin, E.K. (1988) J. Membr. Biol. 103, 41–53.
[6] Kettenmann, H., Hoppe, D., Gottmann, K., Banati, A. and Kreutzberg, G. (1990) J. Neurosci. Res. 26, 278–287.
[7] Nelson, D.J., Jow, B., Jow, F. (1992) J. Membr. Biol. 125, 207–218.

[8] Nörenberg, W., Gebicke-Haerter, P.J. and Illes, P. (1992) Neurosci. Lett. 147, 171–174.
[9] Fischer, H.G., Eder, C. Hadding, U. and Heinemann, U. (1995) Neurosci. 64, 183–191.
[10] Klinkert, B.E.F., LaBadie, J.H. and Bowers, W.E. (1982) J. Exp. Med. 156, 1–19.
[11] Oi, V.T., Jones, P.P., Goding, J.W., Herzenberg, L.A. and Herzenberg, L.A. (1978) Curr. Top. Microbiol. Immunol. 81, 115–120.
[12] Fischer, H.G., Nitzgen, B., Germann, T., Degitz, K., Däubener, W. and Hadding, U. (1993) J. Neuroimmunol. 42, 87–96.
[13] Hamill, O.P., Marty, A., Neher, E., Sakmann, B. and Sigworth, F.J. (1981) Pflügers Arch. 391, 85–100.
[14] Lewis, R.S. and Cahalan, M.D. (1988) Science 239, 771–775.
[15] Eder, C., Fischer, H.G., Hadding, U. and Heinemann, U. (1995) J. Membr. Biol., in press.