Effect of Malnutrition on $K^+$ Current in T Lymphocytes

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Severe malnutrition in children is frequently associated with infectious diseases. Animal models have been useful for studying the effects of malnutrition. One of the immunosuppressive mechanisms of malnutrition is inhibition of the activation of T lymphocytes. The voltage-dependent K(V) potassium channels are vital for the activation of T lymphocytes. The blockade of K(V) channels inhibits the activation of T lymphocytes. Malnutrition could affect the suitable synthesis of K(V) channels in T lymphocytes, producing changes in the magnitude and/or dependency of the voltage of the $K^+$ current. We reported a significant decrease in the $K^+$ current and activation to a 20 mV more positive membrane potential in T lymphocytes of rats with severe malnutrition. These results indicate that the diminution in the $K^+$ conductance by alteration of K(V) channels in severe malnutrition is one of the mechanisms that inhibit the activation of T lymphocytes.

Human T lymphocytes contain a diversity of ionic channels such as $K^+$ (1, 10, 12, 14), Na$^+$ (8), Ca$^{2+}$ (5, 12, 14) and Cl$^-$ channels (15). In recent years, $K^+$ and Ca$^{2+}$ channels have been widely studied due to the vital functions that they carry out in the activation of T lymphocytes. Briefly, T lymphocytes express several populations of K$^+$ channels such as voltage-gated [K(V)] and calcium-activated [K(Ca)] potassium channels. Two types of K(V) channels exist, KV1.3 (type $n$) and charybdotoxin resistant (31). These two are mainly responsible for maintaining the membrane potential in resting human T lymphocytes (31).

Elevation of intracellular free Ca$^{2+}$ is one of the key triggering signals for T-cell activation by antigens (12, 14). Following the engagement of the T-lymphocyte receptor, Ca$^{2+}$ release from intracellular stores occurs and a signal from depleted stores subsequently activates a transmembrane Ca$^{2+}$ conductance ($I_{Ca^{2+}}$) via a Ca$^{2+}$ release-activated Ca$^{2+}$ channel (12, 32). K$^+$ channels have the ability to modulate the rate of Ca$^{2+}$ entry through Ca$^{2+}$ release-activated Ca$^{2+}$ channel channels by hyperpolarization of the membrane (5, 12, 14).

In other species, mainly in rodents, experiments have demonstrated that K(V) channels are present, i.e., $n$, $n'$, and $l$ (13, 14). Type $n'$ and $l$ channels differ from type $n$ by their slow inactivation and absence of cumulative inactivation. In addition, a K(Ca) channel has been reported (16). The ionic channels in rodent T lymphocytes participate in the activation process in a similar way to the one observed in human T lymphocytes (11, 13, 14).

When the hyperpolarization is avoided by the blocking of the K$^+$ channels with some drug, the maintained increase of Ca$^{2+}$ is interrupted and the activation of T lymphocytes is inhibited. This decrease in K$^+$ conductance affects human T lymphocytes and rodent T lymphocytes similarly (6, 12, 14, 24, 25).

Malnutrition is the most common cause of immunodeficiency worldwide (19). Epidemiological observations have confirmed that infection and malnutrition aggravate each other (4, 27). Epidemiological studies have documented the adverse effect of protein-energy malnutrition on morbidity and mortality. It is now recognized that nutritional deficiency is commonly associated with impaired immune responses (3, 4, 19). Malnutrition has been classified as mild, moderate or severe, according to the degree of weight or height deficit, or altered weight/height ratio (21). Children are the most affected group, and its effects can be particularly devastating in the first few years of life (21).

In young children with protein-calorie malnutrition, that is, both marasmus and kwashiorkor, alterations in a number of immune responses have been documented. These alterations include cell-mediated immunity depression, reduced number of circulating T lymphocytes, particularly CD4$^+$ helper T cells and CD3$^+$ CD25$^+$ T cells, decreased lymphocyte stimulation response to mitogens, altered production of cytokines, lower secretory immunoglobulin A (IgA) antibody response on mucosal surfaces, decreased antibody affinity, phagocyte dysfunction, etc. (3, 19). Experimental animal models mainly using rats and mice have been widely used to study the effects of malnutrition. In animal models of nutritional deficiency produced before gestation, during gestation, and during the lactation period, the adverse effects on immune responses have been analyzed (2, 28).

In severe malnutrition, protein synthesis is affected as much in quality as in quantity. Since ionic channels are proteins encrusted in cellular membrane (7), it is possible that one of the factors that complicate infectious illnesses in malnourished patients is inadequate synthesis of K$^+$ channels in T lymphocytes. If this were the case, a decrease in the magnitude of the K$^+$ current or an alteration in its dependence on the voltage would affect the activation of T lymphocytes.

In this work we studied K$^+$ channels in rat peripheral T
lymphocytes with moderate and severe malnutrition induced during lactation. We found that the magnitude of the K⁺ current is smaller and it activates to a 20 mV more positive membrane potential in rats with severe malnutrition. These alterations are not observed in rats with moderate malnutrition.

**MATERIALS AND METHODS**

**Animals.** Wistar rats from the closed colony bred at the Universidad Autónoma Metropolitana-Iztapalapa were used. Rats were kept under 12-hour light/12-hour dark cycle at 22 to 25°C and 45% relative humidity. Females who had two previous litters were bred in acrylic boxes with Betachips bedding (Northeastern Products, Warrensburg, NY). The nursing mothers were fed a balanced diet for rodents (Purina Mills International, Richmond, VA) and filtered water ad libitum.

**Experimental malnutrition and treatment regimen.** The experiments were performed according to the guidelines for the use of experimental animals of the Autonomous Metropolitan University of Iztapalapa, which are in accordance with those approved by the National Institutes of Health (Bethesda, MD). One-day-old Wistar rats from different litters were randomly assigned to two groups. In the well-nourished group, nursing mothers were each given six to eight pups. In the malnourished group, each nursing mother fed 16 to 17 pups. In the latter group, malnutrition was produced in the nursing pups due to food competition (21). Nursing mothers cannot adequately feed 16 to 17 pups even if milk production is adequate, resulting in lower weight gains in the malnourished group (21).

Rats were weighed throughout the nursing period (1 to 24 days of age). The degree of malnutrition that was induced in the malnourished group was of two types, animals with severe malnutrition (weight deficit greater than 40% of that of well-nourished controls) and animals with moderate malnutrition (weight deficit reached 25 to 40% of that of well-nourished controls). Most of the rats (more than 80%) presented severe malnutrition.

Two groups of rats were studied. Group I was well nourished (control group). This group consisted of rats with normal weights for age and without infectious diseases. Their ages ranged from 16 to 24 days. Group II was malnourished. This group included rats with moderate malnutrition (weight deficit >25% and <40% according to age), and rats with severe malnutrition (weight deficit >40% according to age), both without infectious diseases. Their ages ranged from 16 to 24 days.

Peripheral T lymphocytes were obtained for rats with severe malnutrition, moderate malnutrition, and well nourished (control). An animal was used for each day of experimental work.

**Cell separation.** Approximately 1 to 2 ml of blood was collected from each animal by cardiopuncture with a syringe containing heparin (25 U/ml of blood). In the separation of T lymphocytes, B lymphocytes were separated using nylon wool columns (Robbins Scientific). The purity of the isolated T cells determined by expression of CD3 is greater than 90% (18, 22).

Further selection for T cells during voltage-clamp recording was based on their size (diameter smaller than 8 μm) compared with the monocytes and larger lymphocytes (NK cells) (29, 30). Initially, small T cells were selected by size under phase-contrast microscopy (400× magnification), using a micrometric eyepiece. Later a digital image was taken from small T-lymphocyte set with one high-resolution monochrome camera, model DIC-U (World Precision Instruments). The digital photographs were enlarged to allow accurate measurement of cell diameters. T lymphocytes with a diameter of ≥8 μm were rejected. All experiments were performed at room temperature (22 ± 2°C).

**Electrophysiology.** Currents were recorded in a whole-cell patch-clamp configuration. Recordings were made in a chamber continuously perfused with a normal Ringer solution containing 160 mM NaCl, 4.5 mM KCl, 2 mM CaCl₂, 1 mM MgCl₂, and 5 mM HEPES, adjusted to pH 7.4 with NaOH. Pipettes (World Precision Instruments; PG52165-4) with a resistance of 4 to 6 MΩ were filled with a solution containing 140 mM KCl, 10 mM NaCl, 1 mM MgCl₂, 1 mM CaCl₂, 11 mM EGTA, and 10 mM HEPES, adjusted to pH 7.2 with KOH; the free Ca²⁺ concentration was less than 3 nM (9, 17). Equimolar amounts of ions were used in the ion substitution experiments.

Solutions were passed through 0.20-μm filters (Corning). Junction potential between pipette and Ringer solutions was compensated before establishing the seal. Data were collected using an Axopatch 200 A (Axon Instruments, CA) amplifier. Currents were filtered (low-pass filter 5 KHz [3 dB]). In order to generate a set of pulses and also to acquire and analyze the signals, the pClamp software program and a Digidata conversion card were used (Axon Instruments, CA). Before analysis, the digitized records were additionally filtered by low-pass digital filters. Linear capacitive components and leakage currents were subtracted by the scaling of a 20-mV negative pulse (average of five pulses). Some T lymphocytes did not display a linear behavior in the current when the negative pulse of subtraction was applied. In these cases, the leak current was eliminated digitally.

Due to the fact that the ionic current was of small magnitude, in most cases, series resistance was not compensated. Whole-cell currents were evoked by 2,000-ms depolarizing voltage steps from a holding potential of ~80 mV. Pulses were applied at 15- or 20-second intervals. Since the properties of the K⁺ current vary during the first minutes after breaking into the cell (1), at least 7 min were allowed for currents to stabilize. Where appropriate, data are cited as mean ± standard deviation. The means were compared using the two-tailed Student’s t test.

**RESULTS**

Initially the outward current on T lymphocytes from control rats was obtained. T lymphocytes were polarized to a holding potential of ~80 mV. Lengthy depolarizing pulses were applied to evoke the K⁺ current of the steady state. Two second pulses were applied in 20-mV increases. Figure 1a shows typical whole-cell patch-clamp outward currents on resting peripheral T cells. As the depolarization increases, so does the outward current. The activation of the outward current follows a slow temporary course and does not present inactivation.

In order to verify that outward current was due to K⁺, the K⁺ of the pipette solution was replaced by Cs⁺. In Fig. 1b it is observed that when a protocol of pulses equal to the one described in Fig. 1a is applied the outward current is negligible. These results indicate that the outward current was composed fundamentally of K⁺ (7).

In order to observe the effect of the severe malnutrition on the K⁺ current, peripheral T lymphocytes of rats with severe malnutrition were used. Under these conditions, using the same protocol of pulses indicated in Fig. 1, K⁺-evoked currents were similar to the ones observed in T lymphocytes of control rats. However, the magnitude of the outward current was smaller, as is shown in Fig. 2a. In other T lymphocytes a greater effect of the severe malnutrition was observed, they did not present a K⁺ current as can be seen in Fig. 2b. The absence of K⁺ current is similar to the results obtained when K⁺ was replaced by Cs⁺ (Fig. 1b) in the control experiments; in its place, a negative inward current is observed.

The mean of the magnitudes of the K⁺ currents for each membrane potential was obtained and a comparative analysis between groups was made. In Fig. 3a, a significant diminution in the magnitude of the K⁺ current in T lymphocytes of rats with severe malnutrition with respect to control rats as well as a greater dispersion in the data is evident.

In order to observe the effect of severe malnutrition on the dependency of the voltage of the activation of the K⁺ current, the current-voltage relation was obtained. The K⁺ currents were normalized with respect to the maximum current, which was obtained with a +60-mV membrane potential pulse. The
results are shown in Fig. 3b. In T lymphocytes of control rats, the K⁺ current activated at about −60 mV of membrane potential. However, in T lymphocytes of rats with severe malnutrition the K⁺ current activated at about −40 mV of membrane potential, displacing the current-voltage relation 20 mV toward the right, as can be observed in Fig. 3b.

The effect of severe malnutrition on the K⁺ current could be a specific effect or simply a manifestation of malnutrition without regard to type (classification). To discard this possibility, the effect of moderate malnutrition on K⁺ current in T lymphocytes was observed. Unlike the current observed in T lymphocytes of animals with severe malnutrition, the K⁺ current does not seem to be affected in T lymphocytes of rats with moderate malnutrition. In Fig. 4a, the means of the K⁺ current of the control group and the group with moderate malnutrition are compared. Significant differences are not observed. Also, T lymphocytes always presented a K⁺ current, contrary to the K⁺ current absence observed in some T lymphocytes of animals with severe malnutrition.

Moreover, it is observed that the dependence of the voltage of the activation of the K⁺ current is not affected either, as is shown in Fig. 4b. The current-voltage curves of the K⁺ current in T lymphocytes of the control group and those of the group with moderate malnutrition are similar.

**DISCUSSION**

Malnutrition was produced in nursing pups by food competition, using a model previously developed in the laboratory (21). The degree of malnutrition that was obtained was moderate to severe. The analysis of the K⁺ currents in peripheral T lymphocytes was made the same day as their purification, to

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**FIG. 1.** Potassium current in resting rat peripheral T lymphocytes. (a) The solution of the bath contained normal Ringer, and the solution of the pipette contained K⁺ and low Ca²⁺. (b) The K⁺ of the solutions of the bath and the pipette was replaced by Cs⁺, and the outward current is negligible. The holding potential was −80 mV. Outward currents were evoked by depolarizing voltage steps, depolarized to various potentials ranging from −60 to +40 mV in 20-mV steps. The scale of current and time is the same in a and b. The horizontal line indicates zero current.

**FIG. 2.** Currents in resting T lymphocytes of rats with severe malnutrition. In panel a, some T lymphocytes presented a K⁺ current but of smaller magnitude than that observed in Fig. 1a. In panel b, other T lymphocytes did not display K⁺ currents; in their place, an inward current was observed. The protocol of pulses was the same as in Fig. 1. The scale of current and time is the same as in Fig. 1 to emphasize the differences.
avoid using enriched culture medium that could affect T lymphocytes of rats with malnutrition (4, 20). Previous studies have shown different responses in peripheral T cells when nonautologous plasma is used (4, 20). Therefore, autologous plasma was used in the incubation stage for monocyte elimination.

In contrast to the K+ current in human peripheral T lymphocytes, which activate quickly (1), in rat T lymphocytes the activation of the K+ current presents a slower temporary course. Therefore, voltage-clamp pulses of 2,000 ms are applied for obtaining the K+ current in the steady state. The outward current is fundamentally of K+ (13, 14), as can also be inferred by a lack of cumula-

FIG. 3. Mean of the magnitudes of the potassium currents for each membrane potential in T lymphocytes of animals with severe malnutrition. In panel a, K+ current for different membrane potentials, the bars on the left (C) were obtained from T lymphocytes of the control group (n = 12), and those on the right (SM) correspond to T lymphocytes of rats with severe malnutrition (n = 9). In panel b, the K+ current was normalized with respect to the maximal current (Im/Imax) that was obtained with a +60-mV membrane potential pulse. The asterisks (*) and **) indicate P values of <0.005 and <0.001, respectively.

FIG. 4. Absence of effect of moderate malnutrition on potassium current in T lymphocytes. (a) The bars indicate the average K+ current that was obtained from different membrane potentials; significant differences were not observed between the control group (C) and that with moderate malnutrition (MM) (n = 7) (P > 0.05). In panel b, the current-voltage relationship of the K+ current of T lymphocytes from the control group and rats with moderate malnutrition is similar; the K+ currents were normalized as was described for Fig. 3b.
tive inactivation during repetitive depolarizations and low sensitivity to external tetraethylammonium.

The mean magnitude of the K⁺ current in T lymphocytes of rats with severe malnutrition is smaller to the one observed in T lymphocytes of the control group. This is a significant difference for different membrane potentials. It tends to diminish in very positive membrane potentials. On the other hand, the largest standard deviation observed in the mean K⁺ current from T lymphocytes of rats with severe malnutrition is due to the variable decrease in the magnitude of the K⁺ current. Some T lymphocytes only presented a decrease in the magnitude of the K⁺ current, while other T lymphocytes did not exhibit K⁺ current.

In order to observe the effect of the malnutrition on the dependency of the voltage of the opening of the K⁺ channels, the K⁺ currents of each T lymphocyte were normalized with respect to the maximal K⁺ current. This way, the dependency of the voltage of the K⁺ current can be observed without the effect of the current’s magnitude. This is indispensable because of the smaller magnitude of the K⁺ current in rat T lymphocytes with severe malnutrition. In this case, the K⁺ current activated at about −40 mV of membrane potential, with a displacement of 20 mV towards the right of the current-voltage relation with respect to T lymphocytes of the control group. This result is similar to that obtained in T lymphocytes from old men (23).

Different results were obtained in T lymphocytes from rats with moderate malnutrition. The magnitude of the K⁺ current with respect to the membrane potential, as well as the current-voltage relation, was not affected. What this indicates is a specific effect of severe malnutrition on the K⁺ current.

The K(Ca) and K(V) channels play an important role in the process of activation of T lymphocytes (12, 14). The inhibition of the activation of T lymphocytes can take place by blocking some of these subpopulations of K⁺ channels. When K(V) channels are blocked with drugs that diminish K⁺ conductance, the activation of T lymphocytes is inhibited (6, 24, 25, 26). In this work, the study of the K(V) channels indicates that T lymphocytes of rats with severe malnutrition present a smaller K⁺ conductance to different membrane potentials. This smaller K⁺ conductance is due to a diminution in the magnitude of the K⁺ current as well as by displacement towards the right of the normalized current-voltage relation. This diminution in K⁺ conductance can be critical for a suitable activation of T lymphocytes. The T lymphocytes that presented negligible K⁺ currents would be the most affected.

Extrapolating these results to the human case would lead us to conclude that diminution in the K⁺ conductance could be one of the factors that contribute to a greater susceptibility to suffer from and to complicate the pathological processes of infections. It would be one of the mechanisms of immunodeficiency observed in children with severe malnutrition (3, 4, 20). On the other hand, the results for the group with moderate malnutrition indicate that K⁺ conductance is not affected and would not be a factor that limited the activation of T lymphocytes.

In this first stage, we studied the effect of malnutrition on the K⁺ current in rat T lymphocytes. The diseases associated with malnutrition in humans (4, 19) can complicate the study of the effect of severe malnutrition on the K⁺ current. However, these results can serve as a reference.

In summary, in animals with severe malnutrition, the magnitude of the K⁺ current is smaller or it is absent altogether. The current-voltage relation is displaced toward the right by 20 mV. These alterations are not observed in animals with moderate malnutrition. The decrease in K⁺ conductance could be one of the factors that contribute to immunodeficiency in severe malnutrition.

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