T-kininogen, a cystatin-like molecule, inhibits ERK-dependent lymphocyte proliferation

Claudio Acuña-Castillo [a], Mauricio Aravena [a], Elías Leiva-Salcedo [a], Viviana Pérez [a], Christian Gómez [a], Valeria Sabaj [a], Sumiyo Nishimura [a], Claudio Pérez [a], Alicia Colombo [a], Robin Walter [a,b,c], Felipe Sierra [a,b,c,*]

[a] *Instituto de Ciencias Biomédicas, Programa de Biología Celular y Molecular, Facultad de Medicina, Universidad de Chile, Independencia 1027, Santiago, Chile*

[b] *Centro FONDAP de Estudios Moleculares de la Célula, Facultad de Medicina, Universidad de Chile, Independencia 1027, Santiago, Chile*

[c] *Lankenau Institute for Medical Research, 100 Lancaster Avenue, Wynnewood, PA 19096, USA*

Abstract

Plasma levels of kininogens increase with age in both rats and humans. Kininogens are inhibitors of cysteine proteinases, and filarial cysteine proteinase inhibitors (cystatins) reduce the proliferation of T cells. We evaluated whether T-kininogen (T-KG) might mimic this effect, and here we present data indicating that exposure of either rat splenocytes or Jurkat cells to purified T-KG results in inhibition of both ERK activation and [^{3}H]-thymidine incorporation, both basal and in response to ConA or PHA. Interestingly, T-KG did not impair [^{3}H]-thymidine incorporation in response to IL-2, which requires primarily the activation of the JNK and Jak/STAT pathways. These effects were neither the consequence of increased cell death, nor required the activity of kinin receptors. Furthermore, when T cell receptor proximal events were bypassed by the use of PMA plus Calcium ionophore, T-KG no longer inhibited ERK activation, suggesting that inhibition occurs upstream of these events, possibly at the level of membrane associated signal transduction molecules. We conclude that, like filarial cystatins, T-KG inhibits ERK-dependent T cell proliferation, and these observations suggest a possible role for T-KG in immunosenescence.

Keywords: T lymphocytes; Cystatins; T-kininogen; Signal transduction; Cell proliferation

1. Introduction

We have previously described that serum levels of T-kininogen (T-KG), a rat cysteine proteinase inhibitor, increase dramatically during the last few months of life (Walter et al., 1998). Furthermore, expression of T-KG in mouse fibroblasts results in inhibition of both ERK activity and cell proliferation (Torres et al., 2001). We reasoned that the age-related increase in T-KG could potentially play a role in the process of immune senescence, since cysteine proteinase inhibitors can modulate the activity of several components of the immune response. For example, the allergic lung inflammation that characterizes a mouse model of human asthma can be inhibited by the use of an extracellular cysteine protease inhibitor, E64 (Layton et al., 2001). Furthermore, several parasitic worms are capable of using cysteine proteinase inhibitors (generically called filarial cystatins) as a mechanism to avoid and escape the immune system of their host. Hartmann et al. (1997) reported that supernatants from *A. vitae* can inhibit CD3-induced proliferation, and this effect is due to the filarial

Abbreviations: ERK, extracellular signal regulated kinase; MAP kinase, mitogen activated protein kinase; MKP, MAP kinase phosphatase; TdR, thymidine; T-KG, T-kininogen; PARP, poly ADP-ribose polymerase

* Corresponding author at: Biology of Aging Program, National Institute on Aging, National Institutes of Health, 7201 Wisconsin Avenue, Suite 2C231, USA. Tel.: +1 301 496 6402; fax: +1 301 402 0010.

E-mail address: sierraf@nia.nih.gov (F. Sierra).

cystatin Av17. Similarly, cystatins from *O. Volvulus, N. brasilensis* and *L. sigmodonitis* inhibit CD3-induced proliferation of white blood cells and splenocytes (Dainichi et al., 2001; Schönemeyer et al., 2001; Pfaff et al., 2002). The effect of cystatins on the immune response is not restricted to T cell proliferation, since it has been shown that parasitic cystatins can change the patterns of Th1 and Th2 responses in vitro. In vivo, chronic filarial parasitic infections have also been associated with a switch to a Th2 response in the host, apparently as a result of filarial cystatin activity (Vray et al., 2002; Schierack et al., 2003). In summary, filarial cystatins can inhibit the host's response by inhibiting CD3-induced proliferation, and might have additional roles as modulators of the secondary immune response.

We hypothesized that T-KG could affect the immune response of aged individuals in a manner similar to that observed for filarial cystatins. We decided to test this hypothesis in T lymphocytes. Since these cells do not normally produce the protein, we mimicked the in vivo situation by adding purified T-KG to lymphocytes in culture, and we measured the effect on basal, ConA-dependent and IL-2-dependent proliferation. Our results indicate that T-KG indeed inhibits both proliferation and activation of the ERK pathway, both in total rat splenocytes and Jurkat cells. Interestingly, this inhibition was only observed under conditions where activation occurs via the ERK pathway, but not by activation through IL-2.

2. Materials and methods

2.1. Purification of T-KG

T-KG was purified from the serum of LPS-treated young (4–6 months old) Brown Norway Katholiek rats by affinity chromatography, its activity was evaluated in a papain inhibitory assay, and the purity of the preparations was evaluated as previously described (Leiva-Salcedo et al., 2002).

2.2. Cell culture

Jurkat and Daudi cells were kept at 0.5–1 $\times$ 10^6 cells/ml in RPMI 1640 supplemented with 10% FBS at 37 °C, 5% CO_2. Prior to experimentation, the medium was replaced by fresh RPMI 1640 containing only 5% FBS for 24 h.

Rat splenocytes were prepared from male Sprague–Dawley rats (4 months old), which were sacrificed by decapitation. The spleens were removed under sterile conditions, disrupted mechanically, and the splenocytes were resuspended in RPMI 1640 medium (Gibco BRL), spun 10 min at 300 $\times$ *g*, and washed with serum-free RPMI medium. Remaining red blood cells were lysed in ACK lysis buffer (Kriusbeek and Shevac, 2000), and cells were plated for 2 h to eliminate adherent cells. Non-adherent cells were kept at 2 $\times$ 10^6 cells/ml in RPMI 1640 supplemented with 10% FBS (CellGro, Gibco BRL) in the presence of penicillin/streptomycin at 37 °C, 5% CO_2, and used within 24 h of isolation. Prior to experimentation, the medium was replaced by fresh RPMI 1640 containing only 5% FBS.

2.3. Stimulation and proliferation assays

Cells (either Jurkat or primary splenocytes) were seeded at 1 $\times$ 10^6 cells/ml, and incubated with T-KG for 24 h (1 μg/ml, unless noted otherwise). After this period, cells were counted in a hemocytometer, the medium was refreshed (with or without fresh T-KG), and cells were stimulated with different mitogens. Aliquots of 100 μl were plated in 96-well flat bottom plates (Nunc, Roskilde, Denmark) at a density of 2 $\times$ 10^5 cells/well. The mitogens used were 25 μg/ml Con A (Sigma), 0.25 μg/ml PHA (Calbiochem) or 100 U/ml IL-2 (Calbiochem). Cells were further cultured for 24 h, and 4 h before the end of this period, 200 nCi of [^{3}H]-TdR (Amersham) were added to each well. Cells were then harvested, and radioactive incorporation was evaluated in a liquid scintillation counter (Tracor Analytic) using scintillation fluid (Ecosint, National Diagnostic). In the case of Jurkat cells, [^{3}H]-TdR incorporation is expressed as fold induction over the basal level observed in the absence of any stimulus. Due to their low level of basal proliferative capacity, incorporation into primary rat lymphocytes is reported as a percent of the maximal stimulation observed in cells not treated with T-KG. Apoptosis was measured by incorporation of propidium iodine and by PARP activation.

2.4. MAPK activity measurement and Western blot analysis

Cells were seeded and treated as before, and total cell extracts were prepared in Laemmli buffer containing 5% β-mercaptoethanol at 80 °C. Total cell extracts were frozen at −20 °C until needed. Western blots were done under standard conditions, and MAPK activity was assessed by reactivity against anti active-ERK antibodies, according to the instructions of the manufacturers (Cell Signaling). As a standardization, membranes were re-probed with an anti-pan ERK antibody that specifically recognizes ERK-1 (Transduction Laboratories), and actin (ICN). Other antibodies evaluated were anti p38 (Cell Signaling), MKP-1 (Santa Cruz Biotechnology) and MKP-2 (Santa Cruz Biotechnology), PARP (Cell Signaling). Immunoreactive bands were visualized using the ECL enhanced chemiluminescence detection system (Amersham, Arlington Heights, IL) by film exposure (Kodak Biomax Light Film, Rochester, NY).

2.5. Data acquisition and processing

Films from Western blots were scanned, and the band of interest was quantified using Scion Image software (Scion

Corp.®). Statistical significance was calculated with a Prism Graph Pad software. Curves were evaluated by the nonparametric Friedman and Quade test, with Dunn post-test. Comparisons between treatments were evaluated using the nonparametric test of Mann–Whitney.

3. Results

3.1. T-KG inhibits basal proliferation of a T cell, but not a B cell lymphoma

To assess the effect of purified T-KG on the proliferative ability of lymphocytes, we first measured the effect of various concentrations of T-KG (0.1–10 μg/ml) on basal TdR incorporation of both a T cell (Jurkat) and a B cell lymphoma (Daudi). The range of T-KG concentrations used is well below the physiological range for this protein in rat serum (estimated at 1255 ± 57 μg/ml, Raymond et al., 1996). Our unpublished results indicate that most of this T-KG is found in complexes, and the amount of free T-KG present in the serum from old rats is within the range

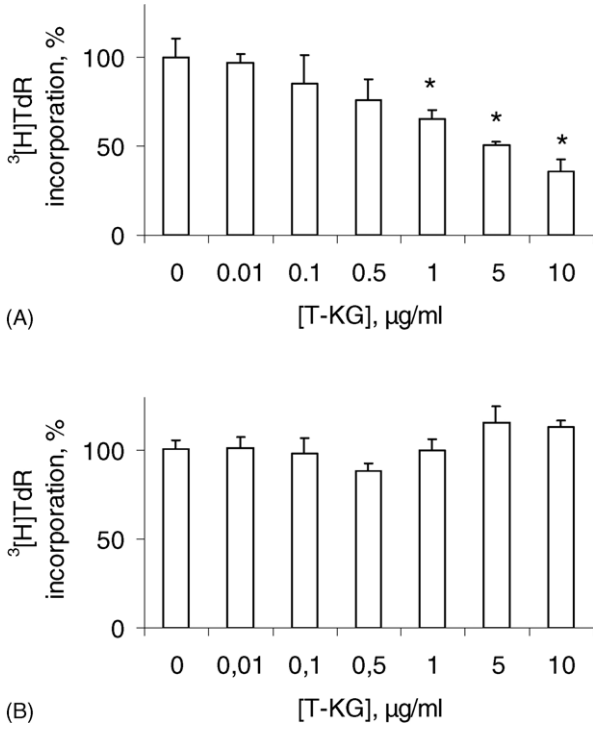

Fig. 1. T-KG inhibits basal incorporation of thymidine in Jurkat cells. Jurkat (A) or Daudi (B) cells (1×10^6 cells/ml) were pre-incubated for 24 h in the presence of different concentrations of T-KG in medium containing 5% FBS. At the end of this period, cells were washed, and resuspended in the same medium and at the same cell density in the presence or absence of T-KG. Incubation was continued for a further 24 h, and [^{3}H]-TdR incorporation was assessed during the last 4 h of this period. Incorporation was normalized against untreated cells from each experiment. Data represents the mean of four independent experiments ± standard error. Asterisk (*) indicates statistical difference from the controls at $p < 0.05$.

used here. Fig. 1A shows that T-KG inhibits TdR incorporation of Jurkat cells in a concentration-dependent manner, with a maximal inhibition greater than 50%. In contrast, TdR incorporation in Daudi cells was not affected by T-KG within the range of concentrations tested (Fig. 1B).

A decreased level of TdR incorporation could be due to either decreased proliferation, or increased cell death. Even though preliminary experiments did not suggest a decrease in the number of viable cells in the presence of T-KG (data not shown), we evaluated cell death directly in Jurkat cells in response to 10 μg/ml T-KG. Fig. 2A indicates that PARP is not proteolytically processed in response to T-KG treatment of Jurkat cells, and a similar result was observed for pro-caspase 3 (data not shown). PARP is cleaved by caspases during almost all forms of apoptosis, and cleavage of pro-caspase 3 is a required step for its activation. Therefore, since these enzymes are not cleaved in the presence of T-KG, we conclude that T-KG does not induce apoptosis in these cells. Furthermore, we did not observe differences between treated or untreated cells when cell death was analyzed by flow cytometry (Fig. 2B). We conclude that T-KG does not induce cell death in Jurkat cells, and thus, the decreased TdR incorporation can be ascribed to decreased proliferation.

T-KG is a precursor of T-kinin. High levels of kinins have been shown to inhibit lymphocyte proliferation (Kimura et al., 1983). To discard the possibility that our results are due to release of kinins from the kininogen precursor during incubation, we tested for the presence of low molecular weight derivatives of T-KG in the culture medium. Release of free T-kinin from T-KG would be expected to reduce the MW of the precursor by 10 kDa, and Fig. 2C indicates that neither the molecular weight nor the amount of full-length T-KG changes during the course of the experiment. Thus, our results indicate that T-KG per se impairs the basal proliferation of Jurkat cells, and that this effect can not be attributed to either apoptosis or release of kinins.

3.2. T-KG inhibits Con A and PHA induced proliferation, but not IL-2-dependent proliferation

Filarial cystatins inhibit the proliferative response of lymphocytes to stimuli. We tested whether T-KG could also inhibit Con A-induced proliferation. For this purpose, Jurkat cells were challenged with various concentrations of T-KG, and Fig. 3A shows that T-KG inhibits Con A-induced proliferation in Jurkat cells. In contrast, T-KG has no effect on the proliferation of these same cells in response to IL-2 (Fig. 3B). Similar experiments were performed with primary rat splenocytes, in which T-KG also inhibited the proliferative response to both Con A and PHA, but not in response to IL-2 (Fig. 4). The results indicate that inhibition of lymphocyte proliferation by T-KG is dependent on the stimulus used, as it only occurs in response to activation via

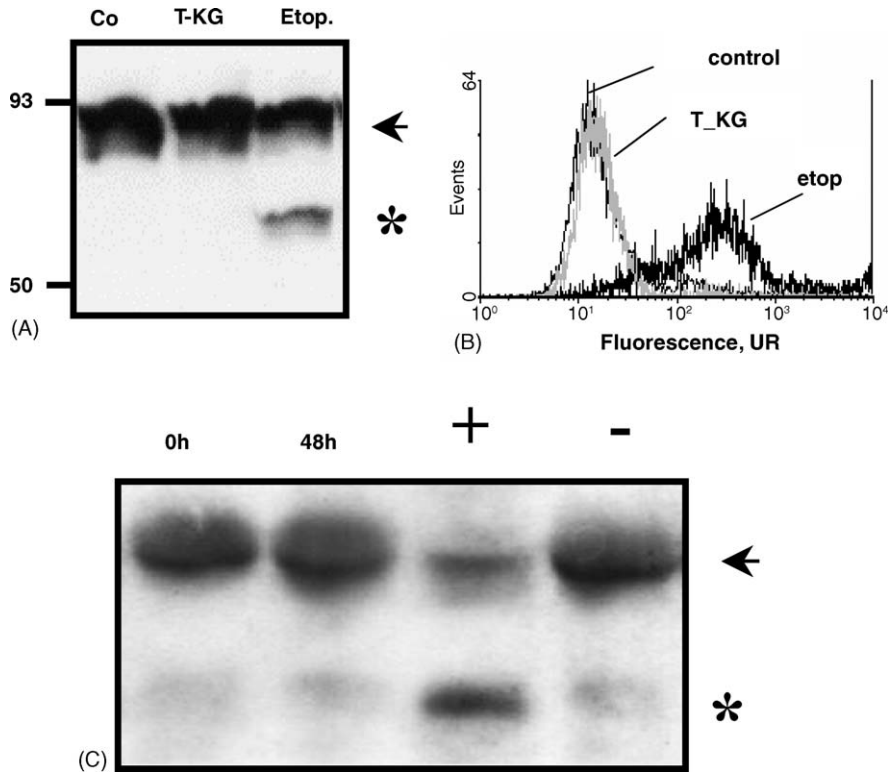

Fig. 2. The inhibitory effect of T-KG is not related to either cell death or the release of kinins. T-KG does not induce cell death in Jurkat cells. (A) Jurkat cells were treated (or not, control) with 10 μg/ml T-KG for 24 h. Total cell extracts were prepared, and cell death was evaluated by Western blot by measuring PARP processing. Lane 1 corresponds to mock samples, lane 2 to extracts derived from T-KG treated cells, and lane 3 is a positive control consisting of cells treated with 25 μM etoposide for 5 h. Full length PARP is indicated by an arrow, while its apoptotic processed form is shown by an asterisk. The position of molecular weight markers is shown on the left. (B) Apoptosis was measured in freshly cultured Jurkat cells, either untreated (control, thin black line), or treated with 10 μg/ml T-KG (T-KG, gray line) or 5 μM etoposide (etop, thick black line) for 24 h. Cells were incubated with propidium iodide in ice-cold buffer for 30 min, and uptake was evaluated using FACS analysis. (C) Kinins are not released from the precursor T-KG during the course of the experiments. The integrity of T-KG was evaluated in the culture medium derived from the same cells used in A. Lane 1 corresponds to a sample obtained immediately after T-KG addition (zero time control). Lane 2 corresponds to the supernatant from cells treated with T-KG for 48 h. Lane 3 is a positive control in which T-KG was digested in vitro with trypsin, while lane 4 shows a negative control in which T-KG was digested in vitro with kallicrein. The kinin moiety is released from T-KG in the presence of trypsin (lane 3), but not kallicrein (lane 4). The arrow corresponds to the position of full length T-KG (68 kDa), while the asterisk denotes the expected position for the heavy chain domain of T-KG if T-kinin release had occurred.

the T cell receptor. More importantly, the results with IL-2 indicate that the basic cell cycle machinery is not impaired in the presence of T-KG.

3.3. The inhibitory effect of T-KG correlates with inhibition of the ERK pathway of signal transduction

Con A treatment of T lymphocytes leads to the activation of a complex array of signal transduction pathways, and crucial among these is the activation of the ERK pathway. Therefore, we chose to study the effect of T-KG on ERK activation in response to Con A treatment of Jurkat cells. Con A induces a strong activation of ERK (both p42 and p44), reaching a maximum at 15 min after Con A administration (Fig. 5A and B). Interestingly, T-KG inhibited maximal ERK activation (40 ± 15 and $52 \pm 17\%$ for ERK-1 and ERK-2, respectively, $p < 0.05$) without affecting the kinetics of induction (Fig. 5A and B). PHA treatment of Jurkat cells also results in a strong activation of ERK, and T-KG exerts a similar inhibitory effect (data not shown). In contrast, T-KG has no effect on the minor level of ERK activation induced by IL-2 (data not shown). We conclude that the effect of T-KG on cell proliferation closely parallels its inhibition of ERK activation. Fig. 5C shows that, as in the case of Jurkat cells, Con A also induces a strong activation of ERK in primary rat splenocytes, and T-KG treatment led to a diminished maximal response of both ERK-1 and ERK-2 (40 ± 15 and $70 \pm 8\%$, respectively).

In fibroblasts expressing T-KG, ERK inhibition occurs via stabilization of both ERK and its phosphatase MKP-1 against proteolytic degradation (Torres et al., 2001). Fig. 6A shows that T-KG does not affect the steady state levels of ERK, JNK, p38, MKP-1 or MKP-2 proteins in Jurkat cells. T-KG treatment does not induce changes in the steady state levels of ERK or MKP-2 in splenocytes either (data not shown). Therefore, ERK inhibition in lymphocytes exposed to exogenous T-KG must occur by a mechanism that is

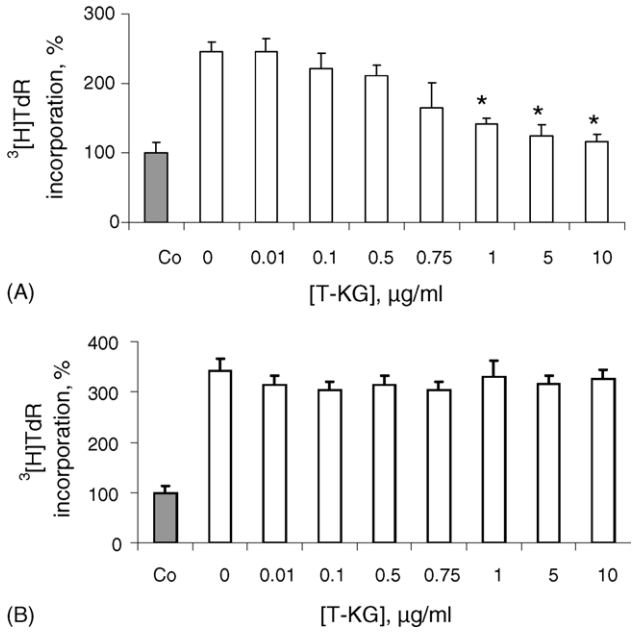

Fig. 3. T-KG inhibits Con A, but not IL-2 induced proliferation of Jurkat cells. Jurkat cells were incubated in the presence or absence of different concentrations of T-KG as described in Fig. 1. After the first 24 h of pre-incubation, cells were stimulated with either Con A (Panel A) or IL-2 (Panel B) for the last 24 h of the experiment. Thymidine incorporation was measured as described in Fig. 1. In both the cases, incorporation was normalized against the value observed in unstimulated cells (Co, gray bar). Data represents the mean ± standard error of four independent experiments, each measured in triplicate. Statistical difference was evaluated by comparison with cells stimulated with Con A but not treated with T-KG (sample labeled as Co, gray bar). Asterisk (*) indicates $p < 0.05$.

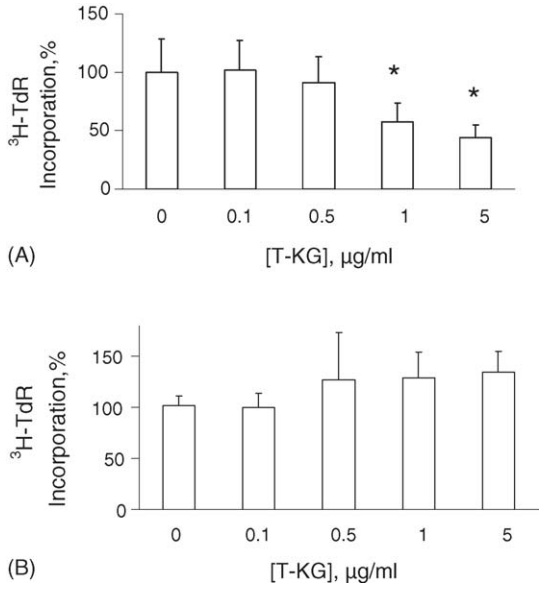

Fig. 4. T-KG inhibits Con A, but not IL-2 induced proliferation in primary rat splenocytes. The experiments were performed essentially as described in Fig. 1 except that 2×10^6 cells/ml were used, and since these cells do not proliferate in the absence of a stimulus, their concentration was not re-adjusted prior to the stimulation with either Con A (Panel A) or IL-2 (Panel B). In this experiment, results were normalized relative to stimulated cells in the absence of T-KG. Each value corresponds to the mean ± standard error of three (Panel A) or two (Panel B) independent experiments done in triplicate. Asterisk (*) indicates statistical difference from the controls at $p < 0.05$.

different from what we have observed in T-KG -expressing fibroblasts.

3.4. The inhibitory effect of T-KG occurs early within the TCR signal generation pathway

Since inhibition of ERK activity does not appear to occur via an imbalance between kinases and phosphatases at the level of ERK itself, we decided to test whether the effect of T-KG was located further upstream in the pathway. For this, we bypassed the early, receptor- associated events in ConA-driven stimulation by using PMA and calcium ionophore, which mimic TCR activation, but act directly at the level of Raf-1 phosphorylation (Rusanescu et al., 2001). This approach has been extensively used to avoid the first steps in signaling associated directly with CD3. In Jurkat cells, PMA plus calcium ionophore induced a biphasic curve of ERK activation, with maximal at 2.5 and 15 min post-induction (Fig. 6B). Interestingly, T-KG had no effect on ERK activation in response to PMA/calcium ionophore. Similar results have been confirmed in splenocytes (data not shown). These results suggest that the inhibitory effect of T-KG in T cells occurs by a mechanism that affects early events within the TCR signal generation pathway.

4. Discussion

In this report, we establish that T-KG inhibits both basal and ConA-dependent proliferation in both Jurkat and primary rat splenocytes. A similar degree of inhibition has been reported to occur in the presence of filarial cystatins, a process that significantly affects the pathogenesis of the organisms involved. The effect was specific to T cell receptor-initiated signaling events, since no decrease in thymidine incorporation was observed when IL-2 was used as a mitogen.

Since T-KG exerts its inhibitory effects even in non-induced Jurkat cells, it is unlikely that the results observed are due to lectin (PHA and Con A) sequestration by T-KG. Furthermore, all experiments were performed in the presence of 5% serum, thus providing ample other sources of possible lectin binding proteins. Induced proliferation and ERK activation in response to the lectins was observed as expected under these conditions in the absence of T-KG. The effect was associated with an inhibition of maximal ERK activation, and this is consistent with the lack of effect of T-KG on IL-2-induced proliferation. Indeed, activation of the ERK pathway has been proposed to be synergistic, but not essential for the IL-2 proliferative response (Ellery and Nicholls, 2002). This same lack of an effect when IL-2 was used as a mitogen allows us to conclude that T-KG does not interfere with the basic cell cycle machinery.

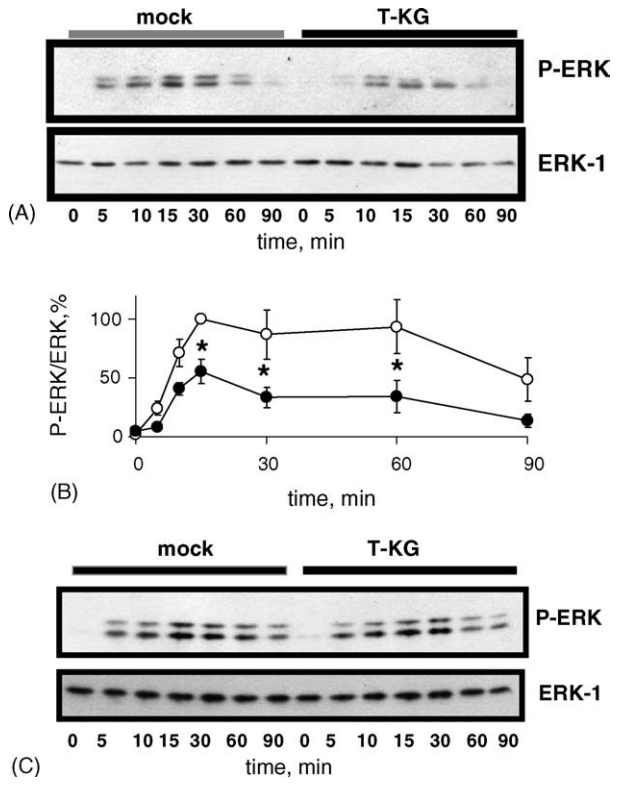

Fig. 5. T-KG inhibits Con A-induced ERK activation in Jurkat cells and rat splenocytes. Jurkat cells (1×10^6 cells/ml, Panels A and B) or rat splenocytes (5×10^6 cells/ml, Panel C) were treated as described in Figs. 4 and 3, respectively, except that total cellular extracts were prepared at the times indicated after addition of the proliferative stimulus. Panel A shows a representative Western blot (from a single set of experiments) obtained after various times of induction of Jurkat cells with Con A. Lanes 1–7 represent mock treated cells, while lanes 8–14 represent cells pre-treated with 1 μg/ml T-KG for 24 h. The top part of Panel A shows active ERK, in the form of P-ERK immunoreactivity, while the lower part shows ERK-1 in the same membrane. Five individually paired experiments were quantified independently for phospho-ERK and the data was normalized against ERK-1 (Panel B). The values at each time point were normalized against the value obtained in mock cells 15 min post-stimulation, which corresponds to maximal activation (100%). Open circles represent mock treated cells, and closed circles represent T-KG treated cells. Panel C shows a representative Western blot showing the effect of T-KG on Con A-induced ERK activity in rat splenocytes. Lanes 1–7 represent mock cells, while lanes 8–14 represent cells pre-treated with 1 μg/ml T-KG.

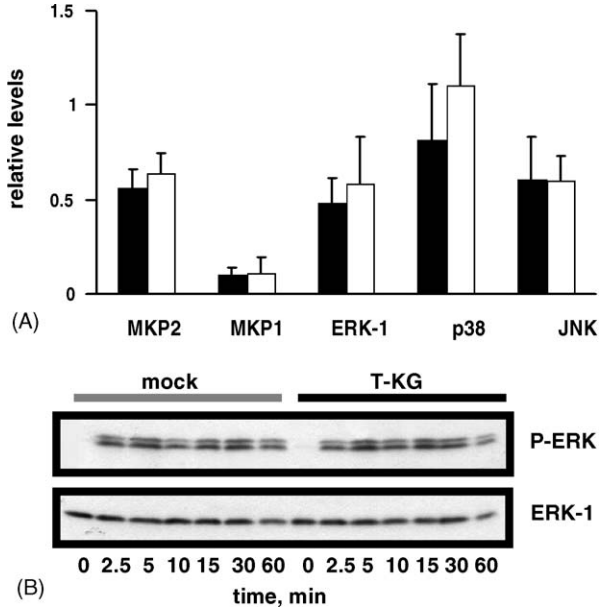

Fig. 6. The inhibitory effect of T-KG is not related to changes in MAPK protein levels, and is located upstream of Raf phosphorylation. (A) T-KG does not change the steady state levels of either MAPKs or their phosphatases in Jurkat cells. We evaluated the steady state levels of MKP-2, MKP-1, ERK, p38, and JNK in Jurkat cells treated with 1 μg/ml T-KG for 24 h. White bars correspond to mock-treated cells and black bars correspond to T-KG treated cells. The densitometric values obtained were normalized against actin. Each value represents the mean ± standard error of five independent experiments. (B) PMA/calcium ionophore-induced ERK activation in Jurkat cells at various times after induction, either in the absence (lanes 1–7) or presence (lanes 8–14) of T-KG. The experiments were repeated five times, and a representative Western blot (from a single set of experiments) is presented.

The results described here are somewhat similar to those previously reported by our group when we analyzed the effect of T-KG expression in fibroblast cell lines (Torres et al., 2001). In that model, T-KG expression also leads to inhibition of cell proliferation, apparently mediated by an impairment in ERK activity. However, ERK inhibition in fibroblasts that express T-KG endogenously appears to be the consequence of an increase in the stability of the phosphatase MKP-1 (Torres et al., 2001), while in the present study we did not observe changes in the steady state levels of either MKP-2 (the major MAP kinase phosphatase in lymphocytes, reviewed Pouyssegur et al., 2002) or ERK isoforms in lymphocytes exposed to exogenous T-KG. Furthermore, the experiments with PMA and calcium ionophore suggest that in this case, inhibition of ERK activity occurs further upstream within the pathway of signal transduction. Interestingly, recent results from our laboratory suggest that the effect of T-KG on proliferation depends on the cell type under study. Indeed, proliferation is inhibited both in over expressing fibroblasts (Torres et al., 2001) and in lymphocytes exposed to exogenous T-KG (this report). In contrast, exogenous T-KG actually induces robust proliferation, as well as ERK activation, both in fibroblasts (Aravena et al., 2005) and in endothelial cells (V.P. et al., submitted).

The precise mechanism used by T-KG to inhibit cell proliferation in lymphocytes is not clear. However, we have discarded the possible involvement of kinins or apoptosis. Therefore, we consider it likely that the mechanism depends on the cysteine proteinase inhibitory properties of the molecule. In this regard, it is interesting to note that the effects of T-KG strongly resemble observations reported for filarial cystatins, a pathogenic form of cysteine proteinase inhibitors. In these cases, pathogenesis often requires inhibition of the immune response of the host. The filarial worms accomplish this by expressing potent cystatins, which inhibit T cell proliferation, and induce a switch from a type 1 (Th1) to a type 2 (Th2) response (Glaser et al., 2001; Sandmand et al., 2002). Our results show that T-KG acts

directly to inhibit T cell proliferation. This has not been previously shown for filarial cystatins. However, our results do not rule out other possible effects of T-KG on antigen processing and presentation, or on cytokine production by macrophages, processes that have been shown to be affected by filarial cystatins (Dainichi et al., 2001; Manoury et al., 2001; Schönemeyer et al., 2001). Our results are particularly relevant in rats, since serum levels of T-KGs increase under at least two different physiological conditions: the acute phase response and aging (Anderson and Heath, 1985; Sierra, 1995). In both cases, the increase in T-KG expression is accompanied by immunosuppression, characterized by decreased T cell proliferation and a switch in the immune response from type 1 (Th1) to type 2 (Th2), similar to what has been described for filarial cystatins. Furthermore, at least in the case of aging, it has been shown that some of the major defects in lymphoproliferation occur at the level of the TCR-linked signal transduction cascade, upstream of Raf-1 (Kirk and Miller, 1998; Pahlavani, 1998; Tinkle et al., 1998; Li et al., 2002). Interestingly, T-KG does not seem to perturb the proliferation of B cells. Interestingly, while changes in subsets of B cell precursors with aging have been documented (Van der Put et al., 2003), most of the decline in immune function with age appears to be due to defects in the T cell lineage (Miller, 2000). The data presented, together with data in the literature concerning aging of the immune system, suggest that the increase in serum T-KG levels during aging could well play a significant regulatory role in the process of immunosenescence, as well as in the age-related switch from Th1 to Th2 responses.

Acknowledgements

The authors want to thank Drs. Andrew Quest, Claudio Torres, Mónica Imarai, and Katherine Marcelain for helpful discussions and for critical comments on the manuscript. This research was funded by FONDECYT (grants 2010071 and 1010615), FONDAP 15010006 and NIH/NIA (R01 AG 13902).

References

Anderson, K.P., Heath, E.C., 1985. The relationship between rat major acute phase protein and the kininogens. J. Biol. Chem. 260 (22), 12065–12071.

Aravena, M., Pérez, C., Pérez, V., Acuña-Castillo, C., Gómez, C., Leiva-Salcedo, E., Nishimura, S., Sabaj, V., Walter, R., Sierra, F., 2005. T-Kininogen can either induce or inhibit proliferation in Balb/C 3T3 fibroblasts, depending on the administration route. Mech. Ageing Dev. 126, 399–406.

Dainichi, T., Maekawa, Y., Ishii, K., Zhang, T., Nashed, B.F., Sakai, T., Takashima, M., Himeno, K., 2001. Nippocystatin, a cysteine protease inhibitor from Nippostrongylus brasiliensis, inhibits antigen processing and modulates antigen-specific immune response. Infect. Immun. 69, 7380–7386.

Ellery, J.M., Nicholls, P.J., 2002. Alternate signalling pathways from the interleukin-2 receptor. Cytokin. Growth Factor Rev. 13, 27–40.

Glaser, R., MacCallum, R.C., Laskowski, B.F., Malarkey, W.B., Sheridan, J.F., Kiecolt-Glaser, J.K., 2001. Evidence for a shift in the Th-1 to Th-2 cytokine response associated with chronic stress and aging. J. Gerontol. 56A, M477–M482.

Hartmann, S., Kyewski, B., Sonnenburg, B., Lucius, R., 1997. A filarial cysteine protease inhibitor down-regulates T-cell proliferation and enhances interleukin-10 production. Eur. J. Immunol. 27, 2253–2260.

Kimura, Y., Saiga, T., Furuya, M., Fujiwara, H., Norose, Y., Okabe, T., Hida, M., 1983. Suppressive effects of Bradykinin to cellular immune responses in vivo and in vitro. Adv. Exp. Med. Biol. 156, 755–766.

Kirk, C.J., Miller, R.A., 1998. Analysis of Raf-1 activation in response to TCR activation and costimulation in murine T-lymphocytes: effect of age. Cell Immunol. 190, 33–42.

Kriusbeek, A.M., Shevac, E., 2000. In vitro assay for mouse lymphocyte function. In: Coligan, J.E., Kruisbeek, A.M., Margulies, D.H., Shevach, E.M., Stober, W. (Eds.), Current Protocols in Immunolgy. John Wiley and Sons Inc., NIH New York (Chapter 3).

Layton, G.T., Harris, S.J., Bland, F.A., Lee, S.R., Fearn, S., Kaleta, J., Wood, M.L., Bond, A., Ward, G., 2001. Therapeutic effects of cysteine protease inhibition in allergic lung inflammation: inhibition of allergen-specific T lymphocyte migration. Inflamm. Res. 50, 400–408.

Leiva-Salcedo, E., Perez, V., Acuña-Castillo, C., Walter, R., Sierra, F., 2002. T-kininogen inhibits kinin-mediated activation of ERK in endothelial cells. Biol. Res. 35, 298–394.

Li, M., Torres, C., Acuña-Castillo, C., Walter, R., Gardner, E.M., Murasko, D.M., Sierra, F., 2002. Defect in ERK2 and p54 (JNK) activation in aging mouse splenocytes. J. Gerontol. A: Biol. Sci. Med. Sci. 57, B41–B47.

Manoury, B., Gregory, W.F., Maizels, R.M., Watts, C., 2001. Bm-CPI-2 a cystatin homolog secreted by the firial parasitc Brugia malayi, inhibits class II MHC-restricted antigen processing. Curr. Biol. 11, 447–451.

Miller, R.A., 2000. Effect of aging on T lymphocyte activation. Vaccine 18, 1654–1660.

Pahlavani, M.A., 1998. T cell signaling: effect of age. Front. Biosci. 3, d1120–d1133.

Pfaff, A.W., Schulz-Key, H., Soboslay, P.T., Taylor, D.W., MacLennan, K., Hoffmann, W.H., 2002. Litomosoides sigmodontis cystatin acts as an immunomodulator during experimental filariasis. Int. J. Parasitol. 32, 171–178.

Pouyssegur, J., Volmat, V., Lenormand, P., 2002. Fidelity and spatio-temporal control in MAP kinase (ERKs) signalling. Biochem. Pharmacol. 64, 755–763.

Raymond, P., Decarie, A., Lantin, F., Raut, R., Morais, R., Couture, R., Adam, A., 1996. A role for Bi and B2 kinin receptors in the modulation of T-kininogen during the acute phase response of inflammation. Peptides 17, 1163–1170.

Rusanescu, G., Gotoh, T., Tian, X., Feig, L.A., 2001. Regulation of Ras signaling specificity by protein kinase C. Mol. Cell Biol. 21, 2650–2658.

Sandmand, M., Bruunsgaard, H., Kemp, K., Andersen-Ranberg, K., Pedersen, A.N., Skinhoj, P., Pedersen, B.K., 2002. Is ageing associated with a shift in the balance between Type 1 and Type 2 cytokines in humans? Clin. Exp. Immunol. 127, 107–114.

Schierack, P., Lucius, R., Sonnenburg, B., Schilling, K., Hartmann, S., 2003. Parasite-specific immunomodulatory functions of filarial cystatin. Infect. Immun. 71, 2422–2429.

Schönemeyer, A., Lucius, R., Sonnenburg, B., Brattig, N., Sabat, R., Schilling, K., Bradley, J., Hartmann, S., 2001. Modulation of human T-cell responses and macrophage functions by onchocystatin, a secreted protein of the filarial nematode Onchocerca volvulus. J. Immunol. 167, 3201–3215.

Sierra, F., 1995. Both T- and K-kininogens increase in the serum of old rats but different mechanisms. Mech. Ageing Dev. 84, 127–137.

Tinkle, C.W., Lipschitz, D., Ponnappan, U., 1998. Decreased association of p56lck with CD4 may account for lowered tyrosine kinase activity in mitogen-activated human T lymphocytes during aging. Cell Immunol. 186, 154–160.

Torres, C., Li, M., Walter, R., Sierra, F., 2001. T-kininogen inhibits fibroblast proliferation in the G(1) phase of the cell cycle. Exp. Cell Res. 269, 171–179.

Van der Put, E., Sherwood, E.M., Blomberg, B.B., Riley, R.L., 2003. Aged mice exhibit distinct B cell precursor phenotypes differing in activation, proliferation and apoptosis. Exp. Gerontol. 38, 1137–1147.

Vray, B., Hartmann, S., Hoebeke, J., 2002. Immunomodulatory properties of cystatins. Cell Mol. Life Sci. 59, 1503–1512.

Walter, R., Murasko, D.M., Sierra, F., 1998. T-kininogen is a biomarker of senescence in rats. Mech. Ageing Dev. 106, 129–144.