Original Paper

Intestinal Na⁺ Loss and Volume Depletion in JAK3-Deficient Mice

Anja T. Umbach Dong Luo Shefalee K. Bhavsar Zohreh Hosseinzadeh
Florian Lang

Department of Physiology, University of Tübingen, Germany

Key Words

ENaC • Blood pressure • Aldosterone • Antinatriuresis • Janus kinase

Abstract

Background/Aims: The Janus kinase 3 JAK3 participates in the signaling of immune cells. Lack of JAK3 triggers inflammatory bowel disease, which in turn has been shown to affect intestinal activity of the epithelial Na⁺ channel ENaC and thus colonic sodium absorption. At least in theory, inflammatory bowel disease in JAK3-deficient mice could lead to intestinal salt loss compromising extracellular volume maintenance and blood pressure regulation. The present study thus explored whether JAK3 deficiency impacts on colonic ENaC activity, fecal Na⁺ excretion, blood pressure and extracellular fluid volume regulation. **Methods:** Experiments were performed in gene-targeted mice lacking functional JAK3 (*jak3*^{-/-}) and in wild type mice (*jak3*^{+/+}). Colonic ENaC activity was estimated from amiloride-sensitive current in Ussing chamber experiments, fecal, serum and urinary Na⁺ concentration by flame photometry, blood pressure by the tail cuff method and serum aldosterone levels by immunoassay. **Results:** The amiloride (50 μM)-induced deflection of the transepithelial potential difference was significantly lower and fecal Na⁺ excretion significantly higher in *jak3*^{-/-} mice than in *jak3*^{+/+} mice. Moreover, systolic arterial blood pressure was significantly lower and serum aldosterone concentration significantly higher in *jak3*^{-/-} mice than in *jak3*^{+/+} mice. Both, absolute and fractional renal Na⁺ excretion were significantly lower in *jak3*^{-/-} mice than in *jak3*^{+/+} mice. **Conclusions:** JAK3 deficiency leads to impairment of colonic ENaC activity with intestinal Na⁺ loss, decrease of blood pressure, increased aldosterone release and subsequent stimulation of renal tubular Na⁺ reabsorption.

Copyright © 2013 S. Karger AG, Basel

Introduction

JAK3 (Janus kinase 3), a tyrosine kinase, contributes to the signaling of hematopoietic cell cytokine receptors [1-5]. The kinase further participates in the response to energy

depletion, hypoxia and ischemia-reperfusion [6-9]. JAK3 stimulation fosters lymphocyte proliferation and suppresses lymphocyte apoptosis [10-12]. Along those lines, JAK3 inhibitors trigger lymphocyte apoptosis [13, 14]. In contrast, JAK3 activation fosters dendritic cell apoptosis [15]. A gain of function mutation of JAK3 (^{A572V}JAK3) [16] is associated with acute megakaryoblastic leukemia [17, 18].

JAK3 deficiency has been shown to trigger inflammatory bowel disease [19], which is in turn known to be associated with downregulation of several Na⁺-transporting proteins [20, 21] including the colonic epithelial Na⁺ channel ENaC [22-26]. Downregulation of ENaC in inflammatory bowel disease has been attributed to transcriptional suppression of the channel protein by proinflammatory cytokines such as tumor necrosis factor (TNF)-alpha [25]. Moreover, ENaC activity is decreased in macroscopically non-inflamed colon from patients with Crohn's disease [26]. As ENaC is important for colonic Na⁺ reabsorption [26, 27], the decreased ENaC activity contributes to diarrhea in those patients [26]. Moreover, decreased ENaC transcription and activity contributes to the diarrhea of ulcerative colitis [22, 23]. Decreased ENaC activity is also involved in the diarrhea of interleukin-2-deficient mice [24].

At least in theory, ENaC activity and extracellular volume regulation may be similarly compromised in mice lacking JAK3. However, ENaC activity and Na⁺ metabolism of those mice have never been studied. The present study thus explored whether JAK3 deficiency influences colonic ENaC activity. To this end, experiments were performed in gene-targeted mice lacking functional JAK3 (*jak3*^{-/-}) and in age- and sex-matched wild type mice (*jak3*^{+/+}).

Materials and Methods

Mice

Experiments were performed in female and male gene-targeted mice lacking functional JAK3 (*jak3*^{-/-}) and in corresponding wild type mice (*jak3*^{+/+}) at the age of 12-16 weeks. The mice had free access to water and control food (Ssniff, Soest, Germany) [28].

Ethics Statement

All animal experiments were conducted according to the German law for the welfare of animals and the surgical procedures on the mice were reviewed and approved by the respective government authority of the state Baden-Württemberg (Regierungspräsidium) prior to the start of the study.

Blood pressure

Systolic arterial blood pressure was determined utilizing the non-invasive tail-cuff method (IITC Life Science, Woodlands Hills, CA) [29]. As reviewed earlier [30], the tail cuff approach to determine arterial blood pressure requires certain precautions to reduce the stress of the animals, including appropriate training of the mice over multiple days, prewarming to an ambient temperature of 34°C, as well as measurement in a quiet and semidark environment at a defined day time. All these precautions were taken in the present study. Readings of two days were averaged to obtain the systolic blood pressure for the respective mouse. All recordings and the data analysis were obtained by utilizing a computerized data acquisition system and software (PowerLab 4/26 and chart5; ADInstruments).

Metabolic cages

For evaluation of renal excretion, mice were placed individually in metabolic cages (Techniplast, Hohenpeissenberg, Germany) for 24 hours urine collection as described previously [31, 32]. The mice were maintained on a standard diet and had free access to tap water before the experiment. They were allowed a two day habituation period. Subsequently, urine and feces were collected daily for four days. To assure quantitative urine collection, metabolic cages were siliconized, and urine was collected under water-saturated oil. Feces were collected in separated tubes.

Determination of serum aldosterone as well as serum, urinary and fecal electrolyte concentrations

To collect blood specimen, animals were lightly anesthetized and about 50 - 200 µl of blood was collected

Fig. 1. Transepithelial resistance and amiloride-sensitive current in colonic epithelium from *jak3*^{-/-} and *jak3*^{+/+} mice. A. Original tracings illustrating the effect of test currents (1 μ A) and of amiloride (50 μ M) on transepithelial potential difference across colonic epithelia from a JAK3-deficient mouse (*jak3*^{-/-}, right) and a corresponding wild type mouse (*jak3*^{+/+}, left). B. Arithmetic means $\pm$ SEM ($n = 5$ /group) of resistance of colonic epithelium from JAK3-deficient mice (*jak3*^{-/-}, black bar) and corresponding wild type mice (*jak3*^{+/+}, white bar). C. Arithmetic means $\pm$ SEM ($n = 5$ /group) of amiloride-sensitive current across colonic epithelium from JAK3-deficient mice (*jak3*^{-/-}, black bar) and corresponding wild type mice (*jak3*^{+/+}, white bar). *** $p < 0.001$ indicates significant difference from *jak3*^{+/+} mice.

into serum tubes by puncturing the retro-orbital plexus [33]. The serum aldosterone concentration was determined using a commercial ELISA kit (Alpha Diagnostics International, Texas; USA). Serum and urinary concentrations of Na⁺ were measured by flame photometry (ELEX 6361, Eppendorf, Hamburg, Germany). Serum and urinary creatinine concentrations were assessed utilizing an enzymatic colorimetric method (Labor & Technik, Berlin, Germany). Fecal dry weight was obtained by drying the collected sample at 80°C for three hours. The fecal samples were prepared for determination of electrolyte content by dissolving in nitric acid (0.75 M HNO₃) and 48 hours at 50°C with continuous shaking. The homogenized samples were centrifuged at 3,500 g for 10 min and 1 ml of the supernatants were again centrifuged at 10,000 g for 5 min. Aliquots from the second supernatants were diluted and the Na⁺ content of the supernatant was determined by flame photometry. The measured electrolyte concentrations were calculated to obtain the fecal sodium excretion in μ mol per g of feces excreted within 24 hours.

Ussing chamber experiments

ENaC activity was estimated from the amiloride-sensitive current across the colonic epithelium. After removing the outer serosal and muscular layer of the late distal colon under a microscope, the tissue was placed onto a custom-made mini-Ussing chamber with an opening area of 0.00769 cm². Transepithelial potential difference (V_{te}) was determined continuously and transepithelial resistance (R_{te}) estimated from the voltage deflections (Δ V_{te}) elicited by imposing rectangular test currents of 1 μ A and 1.2-s duration at a rate of 8/min. The serosal and luminal perfusate contained (in mM) 145 NaCl, 1 MgCl, 2.6 Ca-gluconate, 0.4KH₂PO₄, 1.6 K₂HPO₄, 5 glucose. To assess ENaC-mediated current, 50 μ M amiloride (in DMSO; Sigma, Schnelldorf, Germany) was added to the luminal perfusate.

Statistics

Data are provided as means $\pm$ SEM, n represents the number of independent experiments. All data were tested for significance using unpaired Student *t*-test. Only results with $p < 0.05$ were considered statistically significant.

Fig. 2. Fecal Na⁺ excretion in *jak3*^{-/-} and *jak3*^{+/+} mice. Arithmetic means ± SEM (n = 5-6/group) of fecal Na⁺ excretion in JAK3-deficient mice (*jak3*^{-/-}, black bar) and corresponding wild type mice (*jak3*^{+/+}, white bar). ***p<0.001 indicates significant difference from *jak3*^{+/+} mice.

Fig. 3. Blood pressure and serum aldosterone level in *jak3*^{-/-} and *jak3*^{+/+} mice. A. Arithmetic means ± SEM (n = 11-12/group) of systolic blood pressure in JAK3-deficient mice (*jak3*^{-/-}, black bar) and corresponding wild type mice (*jak3*^{+/+}, white bar). B. Arithmetic means ± SEM (n = 9-12/group) of serum aldosterone in JAK3-deficient mice (*jak3*^{-/-}, black bar) and corresponding wild type mice (*jak3*^{+/+}, white bar). ***p<0.001 indicates significant difference from *jak3*^{+/+} mice.

Results

Ussing chamber experiments were performed in order to study colonic ENaC activity in JAK3-deficient mice (*jak3*^{-/-}) and in wild type mice (*jak3*^{+/+}). ENaC activity was estimated from the effect of ENaC blocker amiloride (50 μM) on the transepithelial potential difference and current. As illustrated in Fig. 1, amiloride application was followed by a lumen-positive voltage deflection and a change of current, which was significantly lower in *jak3*^{-/-} mice than in *jak3*^{+/+} mice.

Decreased ENaC activity is expected to compromise colonic Na⁺ reabsorption. Thus, fecal Na⁺ excretion was determined. As shown in Fig. 2, the fecal excretion of Na⁺ was higher in *jak3*^{-/-} mice than in *jak3*^{+/+} mice.

Intestinal Na⁺ loss could result in extracellular volume depletion with decrease of blood pressure and increase in aldosterone release. Thus, blood pressure was determined utilizing the tail cuff method. As illustrated in Fig. 3A, the systolic arterial blood pressure was significantly lower in *jak3*^{-/-} mice than in *jak3*^{+/+} mice. According to ELISA, plasma aldosterone concentration was significantly higher in *jak3*^{-/-} mice than in *jak3*^{+/+} mice (Fig. 3B).

The secondary hyperaldosteronism is expected to impact on renal tubular Na⁺ reabsorption and thus on urinary Na⁺ excretion. We therefore analyzed *jak3*^{-/-} mice and *jak3*^{+/+} mice in metabolic cages. As illustrated in Fig. 4, both absolute (Fig. 4A) and fractional (Fig. 4B) urinary Na⁺ excretion were lower in *jak3*^{-/-} mice than in *jak3*^{+/+} mice.

Discussion

The present study reveals that JAK3 deficiency was followed by a marked decrease of amiloride-sensitive current reflecting ENaC activity in colonic epithelium. Accordingly, the amiloride-induced voltage deflection was significantly smaller in gene-targeted mice lacking functional JAK3 (*jak3*^{-/-}) than in wild type mice (*jak3*^{+/+}). As JAK3 deficiency leads to inflammatory bowel disease [19], which is in turn known to decrease ENaC activity [22-

Fig. 4. Absolute and fractional renal Na⁺ excretion in *jak3*^{-/-} and *jak3*^{+/-} mice. A. Arithmetic means ± SEM (n = 9-10/group) of renal Na⁺ excretion in JAK3 deficient mice (*jak3*^{-/-}, black bar) and corresponding wild type mice

(*jak3*^{+/-}, white bar). ***p < 0.001 indicates significant difference from *jak3*^{+/-} mice. B. Arithmetic means ± SEM (n = 7-9/group) of fractional renal Na⁺ excretion in JAK3-deficient mice (*jak3*^{-/-}, black bar) and corresponding wild type mice (*jak3*^{+/-}, white bar). *p < 0.05 indicates significant difference from *jak3*^{+/-} mice.

26], the inflammatory bowel disease of those animals presumably contributes to or even accounts for the decline of ENaC activity. The present observations do, however, not rule out additional, more direct effects of JAK3 on ENaC. Possibly, further transport systems [20, 21] are similarly impaired in *jak3*^{-/-} mice.

The impaired intestinal Na⁺ absorption led to enhanced fecal Na⁺ output. Apparently, the intestinal Na⁺ loss resulted in extracellular volume contraction with decrease of blood pressure and increase in aldosterone release. In inflammatory bowel disease the effect of aldosterone on intestinal ENaC activity is blunted or even completely lost [22]. Thus, aldosterone release could not restore colonic Na⁺ absorption. However, the increased aldosterone release contributed to or even accounted for the enhanced renal tubular Na⁺ reabsorption resulting in marked antinatriuresis. Again, we cannot rule out that additional JAK3-dependent mechanisms contribute to the antinatriuresis of JAK3 deficient mice.

At least in theory, JAK3 mutations could similarly impact on Na⁺ metabolism in humans. Gain of function JAK3 mutations may lead to malignancy [34, 35] and loss of function JAK3 mutations are associated with severe immune deficiency [5, 35, 36]. Moreover, pharmacological JAK3 inhibition could, similar to genetic knockout of JAK3, impact on intestinal Na⁺ absorption and extracellular volume homeostasis. JAK3 inhibitors are considered for the treatment of malignancy as well as several inflammatory and immunological diseases, such as rheumatoid arthritis, psoriasis, ulcerative colitis and dry eye disease [5, 35, 37, 38]. Moreover, JAK3 inhibitors are considered for treatment of kidney transplant patients [37, 39]. It remains to be shown, whether application of those inhibitors affects Na⁺ metabolism.

Conclusion

JAK3 deficiency led to decreased activity of ENaC and possibly further intestinal and colonic transport systems. The fecal Na⁺ loss presumably caused extracellular fluid volume depletion with decrease of blood pressure, secondary hyperaldosteronism and renal Na⁺ retention.

Acknowledgements

The authors acknowledge the technical support by Elfriede Faber. This study was supported by the Deutsche Forschungsgemeinschaft (FO 695/1-1) and the Open Access Publishing Fund of Tuebingen University.

References

- 1 Cornejo MG, Boggon TJ, Mercher T: JAK3: a two-faced player in hematological disorders. *Int J Biochem Cell Biol* 2009;41:2376-2379.
- 2 Imada K, Leonard WJ: The Jak-STAT pathway. *Mol Immunol* 2000;37:1-11.
- 3 O'Shea JJ, Gadina M, Schreiber RD: Cytokine signaling in 2002: new surprises in the Jak/Stat pathway. *Cell* 2002;109:S121-S131.
- 4 Shuai K, Liu B: Regulation of JAK-STAT signalling in the immune system. *Nat Rev Immunol* 2003;3:900-911.
- 5 Ghoreschi K, Laurence A, O'Shea JJ: Janus kinases in immune cell signaling. *Immunol Rev* 2009;228:273-287.
- 6 Ananthakrishnan R, Hallam K, Li Q, Ramasamy R: JAK-STAT pathway in cardiac ischemic stress. *Vascul Pharmacol* 2005;43:353-356.
- 7 Nagel S, Papadakis M, Pfleger K, Grond-Ginsbach C, Buchan AM, Wagner S: Microarray analysis of the global gene expression profile following hypothermia and transient focal cerebral ischemia. *Neuroscience* 2012;208:109-122.
- 8 Wang G, Qian P, Jackson FR, Qian G, Wu G: Sequential activation of JAKs, STATs and xanthine dehydrogenase/oxidase by hypoxia in lung microvascular endothelial cells. *Int J Biochem Cell Biol* 2008;40:461-470.
- 9 Bhavsar SK, Gu S, Bobbala D, Lang F: Janus kinase 3 is expressed in erythrocytes, phosphorylated upon energy depletion and involved in the regulation of suicidal erythrocyte death. *Cell Physiol Biochem* 2011;27:547-556.
- 10 de Toter D, Meazza R, Capaia M, Fabbi M, Azzarone B, Balleari E, Gobbi M, Cutrona G, Ferrarini M, Ferrini S: The opposite effects of IL-15 and IL-21 on CLL B cells correlate with differential activation of the JAK/STAT and ERK1/2 pathways. *Blood* 2008;111:517-524.
- 11 Fainstein N, Vaknin I, Einstein O, Zisman P, Ben Sasson SZ, Baniyash M, Ben Hur T: Neural precursor cells inhibit multiple inflammatory signals. *Mol Cell Neurosci* 2008;39:335-341.
- 12 Nakayama J, Yamamoto M, Hayashi K, Satoh H, Bundo K, Kubo M, Goitsuka R, Farrar MA, Kitamura D: BLNK suppresses pre-B-cell leukemogenesis through inhibition of JAK3. *Blood* 2009;113:1483-1492.
- 13 Kim BH, Oh SR, Yin CH, Lee S, Kim EA, Kim MS, Sandoval C, Jayabose S, Bach EA, Lee HK, Baeg GH: MS-1020 is a novel small molecule that selectively inhibits JAK3 activity. *Br J Haematol* 2010;148:132-143.
- 14 Uckun FM, Vassilev A, Dibirdik I, Tibbles H: Targeting JAK3 tyrosine kinase-linked signal transduction pathways with rationally-designed inhibitors. *Anticancer Agents Med Chem* 2007;7:612-623.
- 15 Yamaoka K, Min B, Zhou YJ, Paul WE, O'Shea JJ: Jak3 negatively regulates dendritic-cell cytokine production and survival. *Blood* 2005;106:3227-3233.
- 16 Haan C, Rolvering C, Raulf F, Kapp M, Druckes P, Thoma G, Behrmann I, Zerwes HG: Jak1 has a dominant role over Jak3 in signal transduction through gamma-c containing cytokine receptors. *Chem Biol* 2011;18:314-323.
- 17 Malinge S, Ragu C, Della-Valle V, Pisani D, Constantinescu SN, Perez C, Villeval JL, Reinhardt D, Landman-Parker J, Michaux L, Dastugue N, Baruchel A, Vainchenker W, Bourquin JP, Penard-Lacronique V, Bernard OA: Activating mutations in human acute megakaryoblastic leukemia. *Blood* 2008;112:4220-4226.
- 18 Walters DK, Mercher T, Gu TL, O'Hare T, Tyner JW, Loriaux M, Goss VL, Lee KA, Eide CA, Wong MJ, Stoffregen EP, McGreevey L, Nardone J, Moore SA, Crispino J, Boggon TJ, Heinrich MC, Deininger MW, Polakiewicz RD, Gilliland DG, Druker BJ: Activating alleles of JAK3 in acute megakaryoblastic leukemia. *Cancer Cell* 2006;10:65-75.
- 19 Murata Y, Yamashita A, Saito T, Sugamura K, Hamuro J: The conversion of redox status of peritoneal macrophages during pathological progression of spontaneous inflammatory bowel disease in Janus family tyrosine kinase 3(-/-) and IL-2 receptor gamma(-/-) mice. *Int Immunol* 2002;14:627-636.
- 20 Martinez-Augustin O, Romero-Calvo I, Suarez MD, Zarzuelo A, de Medina FS: Molecular bases of impaired water and ion movements in inflammatory bowel diseases. *Inflamm Bowel Dis* 2009;15:114-127.
- 21 Sullivan S, Alex P, Dassopoulos T, Zachos NC, Iacobuzio-Donahue C, Donowitz M, Brant SR, Cuffari C, Harris ML, Datta LW, Conklin L, Chen Y, Li X: Downregulation of sodium transporters and NHERF proteins in IBD patients and mouse colitis models: potential contributors to IBD-associated diarrhea. *Inflamm Bowel Dis* 2009;15:261-274.
- 22 Amasheh S, Barmeyer C, Koch CS, Tavalali S, Mankertz J, Eppe HJ, Gehring MM, Florian P, Kroesen AJ, Zeitz M, Fromm M, Schulzke JD: Cytokine-dependent transcriptional down-regulation of epithelial sodium channel in ulcerative colitis. *Gastroenterology* 2004;126:1711-1720.
- 23 Greig ER, Boot-Handford RP, Mani V, Sandle GI: Decreased expression of apical Na⁺ channels and basolateral Na⁺, K⁺-ATPase in ulcerative colitis. *J Pathol* 2004;204:84-92.

- 24 Barmeyer C, Horak I, Zeitz M, Fromm M, Schulzke JD: The interleukin-2-deficient mouse model. *Pathobiology* 2002;70:139-142.
- 25 Bergann T, Zeissig S, Fromm A, Richter JF, Fromm M, Schulzke JD: Glucocorticoids and tumor necrosis factor- α synergize to induce absorption by the epithelial sodium channel in the colon. *Gastroenterology* 2009;136:933-942.
- 26 Zeissig S, Bergann T, Fromm A, Bojarski C, Heller F, Guenther U, Zeitz M, Fromm M, Schulzke JD: Altered ENaC expression leads to impaired sodium absorption in the noninflamed intestine in Crohn's disease. *Gastroenterology* 2008;134:1436-1447.
- 27 Kunzelmann K, Mall M: Electrolyte transport in the mammalian colon: mechanisms and implications for disease. *Physiol Rev* 2002;82:245-289.
- 28 Alesutan I, Daryadel A, Mohebbi N, Pelzl L, Leibrock C, Voelkl J, Bourgeois S, Dossena S, Nofziger C, Paulmichl M, Wagner CA, Lang F: Impact of bicarbonate, ammonium chloride, and acetazolamide on hepatic and renal SLC26A4 expression. *Cell Physiol Biochem* 2011;28:553-558.
- 29 Ackermann TF, Boini KM, Beier N, Scholz W, Fuchss T, Lang F: EMD638683, a novel SGK inhibitor with antihypertensive potency. *Cell Physiol Biochem* 2011;28:137-146.
- 30 Meneton P, Ichikawa I, Inagami T, Schnermann J: Renal physiology of the mouse. *Am J Physiol Renal Physiol* 2000;278:F339-F351.
- 31 Pathare G, Foller M, Daryadel A, Mutig K, Bogatikov E, Fajol A, Almilaji A, Michael D, Stange G, Voelkl J, Wagner CA, Bachmann S, Lang F: OSR1-Sensitive Renal Tubular Phosphate Reabsorption. *Kidney Blood Press Res* 2012;36:149-161.
- 32 Nasir O, Umbach AT, Rexhepaj R, Ackermann TF, Bhandaru M, Ebrahim A, Artunc F, Kempe DS, Puchchakayala G, Siraskar B, Foller M, Saeed A, Lang F: Effects of gum arabic (*Acacia senegal*) on renal function in diabetic mice. *Kidney Blood Press Res* 2012;35:365-372.
- 33 Tang C, Zelenak C, Volkl J, Eichenmuller M, Regel I, Frohlich H, Kempe D, Jimenez L, Le Bellego L, Vergne S, Lang F: Hydration-sensitive gene expression in brain. *Cell Physiol Biochem* 2011;27:757-768.
- 34 Levine RL: JAK-mutant myeloproliferative neoplasms. *Curr Top Microbiol Immunol* 2012;355:119-133.
- 35 Vainchenker W, Dusa A, Constantinescu SN: JAKs in pathology: role of Janus kinases in hematopoietic malignancies and immunodeficiencies. *Semin Cell Dev Biol* 2008;19:385-393.
- 36 Casanova JL, Holland SM, Notarangelo LD: Inborn Errors of Human JAKs and STATs. *Immunity* 2012;36:515-528.
- 37 Trelinski J, Robak T: JAK inhibitors: pharmacology and clinical activity in chronic myeloproliferative neoplasms. *Curr Med Chem* 2013;20:1147-1161.
- 38 Alicea-Velazquez NL, Boggon TJ: The Use of Structural Biology in Janus Kinase Targeted Drug Discovery. *Curr Drug Targets* 2011;12:546-555.
- 39 Borie DC, Changelian PS, Larson MJ, Si MS, Paniagua R, Higgins JP, Holm B, Campbell A, Lau M, Zhang S, Flores MG, Rousvoal G, Hawkins J, Ball DA, Kudlacz EM, Brissette WH, Elliott EA, Reitz BA, Morris RE: Immunosuppression by the JAK3 inhibitor CP-690,550 delays rejection and significantly prolongs kidney allograft survival in nonhuman primates. *Transplantation* 2005;79:791-801.