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In Vivo Evidence of the Immunomodulatory Activity of Orally Administered *Aloe vera* Gel

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The gels of *Aloe* species contain immunomodulatory components such as aloctin A and acemannan. Most studies on these gels were performed in *in vitro* cell culture systems. Although several studies examined their immunomodulatory activity *in vivo*, the route of administration was intraperitoneal or intramuscular. Here, we evaluated the *in vivo* immunomodulatory activity of processed *Aloe vera* gel (PAG) in mice. Oral administration of PAG significantly reduced the growth of *C. albicans* in the spleen and kidney following intravenous injection of *C. albicans* in normal mice. PAG administration also reduced the growth of *C. albicans* in streptozotocin-induced diabetic mice. PAG administration did not increase ovalbumin (OVA)-specific cytotoxic T lymphocyte (CTL) generation in normal mice, but did increase it in high-fat-diet induced diabetic mice. These findings provide the first clear evidence for the immunomodulatory activity of orally administered *Aloe vera* gel.

Key words: Processed *Aloe vera* gel, Immunomodulation, *C. albicans*, Cytotoxic T lymphocyte

INTRODUCTION

The gels of *Aloe* species contain immunomodulatory polysaccharides, such as acetylated mannan (Yagi et al., 1997; Manna and McAnalley, 1993), glucomannan (Gowda et al., 1979), and galactogalacturan (Mandal and Das, 1980). Acemannan, a mixture of various length polymer chains of β-(1,4)-linked acetylated galactomannan isolated from *Aloe vera*, may be the best-characterized immunomodulatory polysaccharides (Manna and McAnalley, 1993). When administered intraperitoneally to tumor-implanted mice, acemannan completely cured or significantly reduced the tumor burden (Peng et al., 1991). Acemannan is also effective in the treatment of spontaneously developed canine and feline fibrosarcomas (Harris et al., 1991; King et al., 1995). Some of the immunoaugmenting activities of acemannan are mediated through the activation of macrophages. Acemannan activates macrophages to produce inflammatory cytokines such as IL-6 and TNF-a (Zhang and Tizard, 1996), increase NO production by macrophages (Karaca et al., 1995; Ramamoorthy et al., 1996; Djeraba and Quere, 2000), and upregulate the phagocytic and candidicidal activities of macrophages (Stuart et al., 1997). Acemannan also augments hematopoiesis (Egger et al., 1996) and induces maturation of immature dendritic cells (Lee et al., 2001).

The gel of *Aloe vera* is sold commercially and is a common ingredient in a range of healthcare products. Most studies have examined immunomodulatory activity of *Aloe* components in *in vitro* cell culture systems, with *in vivo* studies using intraperitoneal or intramuscular administration. Here, we examined the immunomodulatory activity of orally administered processed *Aloe vera* gel (PAG). PAG-treated or untreated mice were infected with *C. albicans*, and then the number of viable fungi residing in the spleen and kidney was quantified 3 to 7 days later. In addition, PAG-treated or untreated mice were immunized with OVA, and then OVA-specific CTL activity was mea-

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sured via an *in vivo* CTL assay using OVA-peptide pulsed target cells. To the best of our knowledge, this is the first report demonstrating the *in vivo* immunomodulatory activity of orally administered *Aloe vera* gel.

MATERIALS AND METHODS

Processed *Aloe vera* gel

The PAG was provided by Aloecorp. Basic PAG processing involves incubation of *Aloe vera* gel with cellulase, termination of the reaction by heating, passage through a charcoal column to remove anthraquinones and other colored substances, and then ethanol precipitation (Qui et al., 2000). PAG is not a commercial product currently marketed by any company. The total polysaccharide content of the PAG was 23.85% ± 0.35% (based on the dry weight of the residual fraction inside a dialysis sac (MW cut-off, 3,500 daltons) after extensive dialysis against distilled water). The molecular size distribution of the polysaccharides was determined by comparing their retention time with that of broad dextran standards, MW 7.2 kD, 16.23 kD, 230 kD, and 520 kD (Phenomenex Co.), in BiosepSEC columns (S4000 and S3000, Phenomenex). The total polysaccharide content between 5 kD and 400 KD was 20.47%. Endotoxin levels in the PAG were determined at 1000 μg/mL by a limulus amebocyte assay (sensitivity 0.125 ng/mL) (Associates of Cape Cod) and contained less than 0.125 ng/mL. PAG was dissolved in phosphate buffered saline (PBS) just before administration.

Induction of type I diabetes mellitus (T1DM)

Male ICR mice were purchased from the Charles River Laboratory of Animal Science (Orient Co.) at 8 weeks of age and were housed at five per cage in a temperature- and atmosphere-controlled room. T1DM was induced by injecting streptozotocin (40 mg/kg/day) intramuscularly for 5 consecutive days. One week later, blood glucose levels were measured using blood glucose test strips (GlucoDr^{TM}, All Medicus Co. Ltd.). Mice exhibiting blood glucose levels >300 mg/dL were selected as T1DM animals.

Induction of diet-induced obesity (DIO)

Male C57BL/6NCrjBgi mice were purchased from the Charles River Laboratory of Animal Science (Orient Co.) at 4 weeks old, fed a normal diet for 1 week, and then either fed a high-fat diet (OpenSource diets #D12492; Research Diets Inc.) to induce obesity or a regular diet (Open-Source diets #D12450B; Research Diets Inc.). All of the mice on the high-fat diet developed a DIO phenotype and had markedly higher levels of circulating blood glucose than mice on a regular diet. DIO mice exhibiting fasting blood glucose levels above 180 mg/dL were used for subsequent experiments.

Candida infection

Candida albicans NIH A-207 was kindly provided by Professor Suhn Young Im (Chonnam National University). *C. albicans* grown in Sabouraud dextrose broth (BBL Microbiology System) were washed in PBS, diluted in PBS to 1×10^6 cells/mL, and then injected into mice (i.v.) via the tail vein (1×10^5 cells/mouse). On days 3 and 7 after the infection, spleens and kidneys from individual mice were removed aseptically and homogenized in PBS. The number of colony forming units (CFU) in the specimens was determined by dilution plating on Sabouraud dextrose agar (BBL Microbiology System).

In vivo CTL assay

Mice were immunized (i.v.) with soluble OVA (100 μg/mouse), and OVA-specific CTL activity was measured 7 days later. Target cells for *in vivo* evaluation of cytotoxic activity were prepared as described previously (Lee et al., 2007). Briefly, naive C57BL/6 spleen cells were either pulsed with 10^{-6} M OVA[257-264] peptide for 1 h at 37°C and then labeled with a high concentration of CFSE (5 μM), or just labeled with a low concentration of CFSE (1 μM). An equal number of cells from each population were mixed together and injected (i.v.) into immunized recipient mice (1×10^7 cells/mouse). Specific *in vivo* cytotoxicity was determined by flow cytometry for the lymph node and spleen cells isolated from the recipient mice 18 h after i.v. injection. The ratio between the percentages of uncoated vs OVA[257-264]-coated ($CFSE^{low}/CFSE^{high}$) was calculated to obtain a numerical value for cytotoxicity.

Statistical analysis

Data are expressed as mean ± S.D. Statistical significance was assessed by one-way ANOVA followed by a Student's t-test.

RESULTS

PAG administration increases organ clearance of *C. albicans* in normal mice

To examine the immunomodulatory activity of PAG administered orally *in vivo*, nine-week-old normal C57BL/6 mice were orally administered 100, 200, or 400 mg/kg PAG twice a day for four weeks. Then each

mouse was injected with 1×10^5 *C. albicans* intravenously via the tail vein. On days 3 and 7 after the injection, spleen and kidneys were removed aseptically and homogenized in PBS, and the number of CFU in the specimens was determined by dilution plating. Kidneys were much more susceptible than spleen to *C. albicans* infection, reaching 2.9×10^4 CFUs/kidney at day 3 and 4.7×10^4 CFUs/kidney at day 7 in the PBS-treated control mice (Fig. 1). Oral administration of PAG dose dependently and significantly reduced the fungal burden in the kidney and spleen.

PAG administration increases organ clearance of *C. albicans* in T1DM mice

To examine the immunomodulatory activity of PAG in immunosuppressed mice, T1DM was induced in ICR mice by injecting streptozotocin (i.m.) for five consecutive days. One week later, mice exhibiting blood glucose levels of >300 mg/dL were selected as T1DM animals and were orally administered 100, 200, or 400 mg/kg PAG twice a day for four weeks. Then each mouse was injected with 1×10^5 *C. albicans* intravenously via the tail vein. Three days after the injection, the number of CFUs in the spleens and kidneys was determined. PAG administration reduced the fungal burden in the spleens and kidneys of T1DM mice (Fig. 2), but with less efficacy than in normal mice (Fig. 1), perhaps due to the systemic toxic effects of streptozotocin. The number of CFUs in the spleens and kidneys of untreated T1DM mice was much higher than that in the spleens and kidneys of normal mice. PAG did not affect blood glucose in this T1DM mouse model (data not shown).

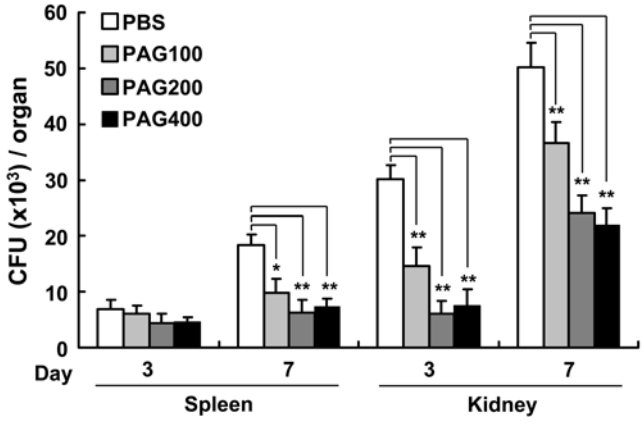

Fig. 1. Effects of PAG administration on the organ clearance of *C. albicans* in normal mice. Normal C57BL/6 mice were orally administered the indicated doses of PAG twice a day for 4 weeks. *C. albicans* grown in Sabouraud dextrose broth were washed in PBS, diluted in PBS, and then injected into mice (i.v.) via tail vein (1×10^5 /mouse). On days 3 and 7 after injection, spleen and kidneys were removed aseptically and homogenized in PBS, and the number of CFU in the specimens was determined by dilution plating. These experiments were repeated three times with five animals per group. Results are mean ± S.D. $*p < 0.05$, $**p < 0.01$ compared with PBS-treated mice.

PAG administration does not increase OVA-specific CTL activity in normal mice

The effects of oral PAG administration on the generation of antigen-specific CTLs were examined in nine-week-old normal C57BL/6 mice. Mice were orally administered different doses of PAG twice a day for four weeks. Then, each mouse was immunized (i.v.) with soluble OVA (100 μg/mouse), and OVA-specific CTL activity was measured 7 days later using an *in vivo* CTL assay. Administration of PAG (up to 100 mg/kg) did not increase OVA-specific CTL activity in normal mice (Fig. 3).

PAG administration increases OVA-specific CTL activity in DIO mice

The effects of oral PAG administration on the generation of antigen-specific CTLs were also examined in DIO mice. DIO was induced in C57BL/6 mice by feeding a high-fat diet for 21 weeks. DIO mice ex-

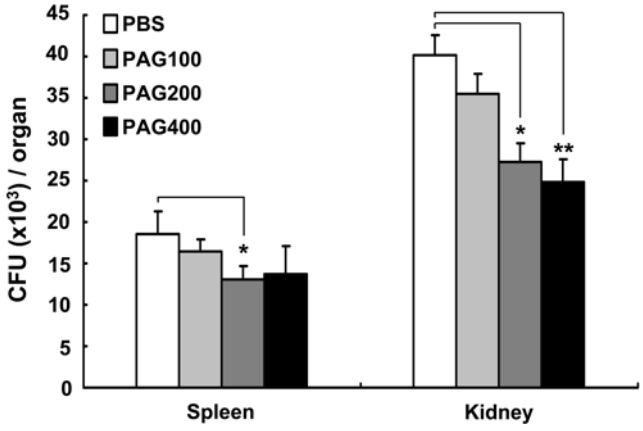

Fig. 2. Effects of PAG administration on the organ clearance of *C. albicans* in T1DM mice. T1DM was induced in ICR mice by injecting streptozotocin (i.m.) for five consecutive days. One week later, mice exhibiting blood glucose levels of >300 mg/dL were selected as T1DM animals and allocated randomly into four groups of five animals per group. After oral administration of the indicated amounts of PAG twice a day for 4 weeks, *C. albicans* was injected into mice (i.v.) via the tail vein (1×10^5 cells/mouse). On days 3 and 7 after injection, spleen and kidneys from individual mice were removed aseptically and homogenized in PBS, and the number of CFU in the specimens was determined by dilution plating. These experiments were repeated three times with five animals per group. Results are mean ± S.D. $*p < 0.05$, $**p < 0.01$ compared with PBS-treated mice.

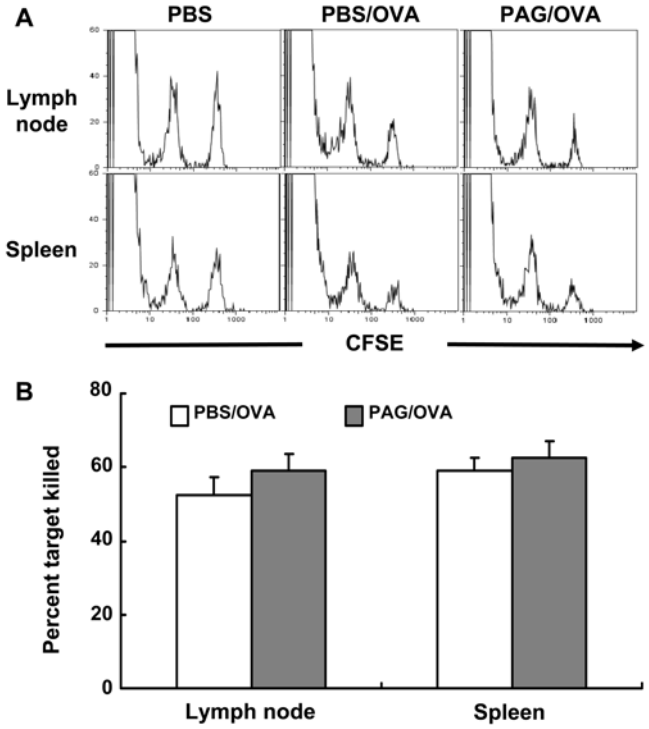

Fig. 3. Effects of PAG administration on the generation of OVA-specific CTL in normal mice. Normal C57BL/6 mice were orally administered 100 mg/kg of PAG twice a day for four weeks. Then, each mouse was immunized with soluble OVA (100 μg/mouse, i.v.). To analyze OVA-specific cytotoxicity, cells pooled from the spleens and lymph nodes of naive syngeneic mice were pulsed with OVA[257-264] peptide and labeled with a high concentration of CFSE ($CFSE^{high}$). To control for antigen specificity, unpulsed syngenic cells were labeled with a low concentration of CFSE ($CFSE^{low}$). A 1:1 mixture of each target cell population was injected (i.v.) into recipient mice and specific cytotoxicity was determined 18 h later. (**A**) Representative histograms of the lymph node cells of individual mice are shown. Percentages of the specific killing of OVA[257-264] peptide-pulsed target cells in the lymph nodes and spleens (**B**) are graphically represented. These experiments were repeated three times with five animals per group.

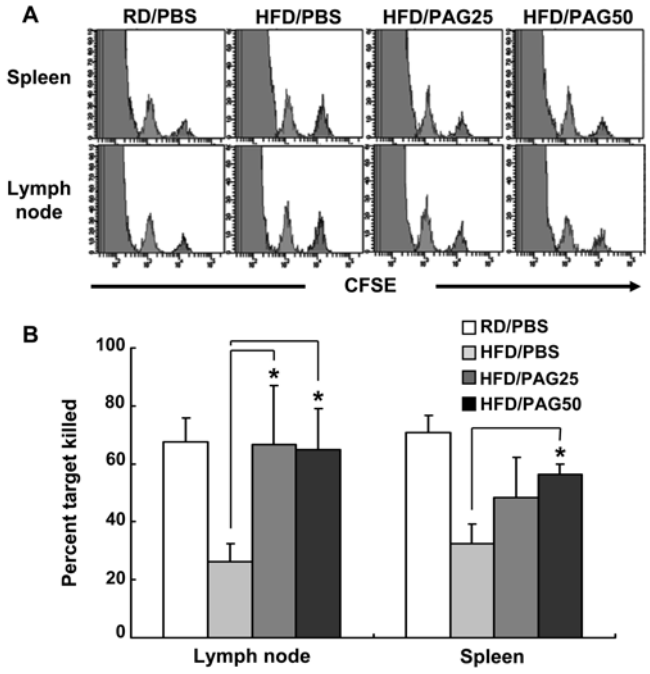

Fig. 4. Effects of PAG administration on the generation of OVA-specific CTL in DIO mice. Male C57BL/6N mice were either fed a high-fat diet (HFD) to induce DIO or a regular diet (RD) for 21 weeks. DIO mice with fasting blood glucose levels >180 mg/dL were selected and allocated randomly into five groups of 18 animals per group. Animals were administered either PBS or PAG (25 and 50 mg/kg, respectively) orally for 8 weeks with free access to the high-fat diet. Mice fed regular diet served as normal controls. Then each mouse was immunized with soluble OVA (100 μg/mouse, i.v.), and OVA-specific cytotoxicity was measured by an *in vivo* CTL assay as described in Fig. 3. (**A**) Representative histograms of the lymph node cells of individual mice are shown. The percentages of specific killing of OVA[257-264] peptide-pulsed target cells in the lymph nodes and spleens (**B**) are graphically represented. Results are mean ± S.D. *$p < 0.05$ compared with HFD-fed PBS-treated mice.

hibiting fasting blood glucose levels above 180 mg/dL were selected, grouped randomly into experimental groups, and then orally administered different doses of PAG for 8 weeks with continued free access to the high-fat diet. Then each mouse was immunized (i.v.) with soluble OVA (100 μg/mouse), and OVA-specific CTL activity was measured 7 days later using an *in vivo* CTL assay. Untreated DIO mice (HFD/PBS) were markedly suppressed in their ability to generate OVA-specific CTL activity compared to regular diet-fed mice (RD/PBS) (Fig. 4). However, oral administration of PAG for 8 weeks restored the suppressed OVA-specific CTL generation in the DIO mice.

DISCUSSION

Previously, we showed that polysaccharides isolated from cellulose-treated *Aloe vera* gel exhibited more potent macrophage-activating activity *in vitro* than polysaccharides isolated from fresh *Aloe vera* gel (Im et al., 2005). Through molecular size-activity relationship studies, we showed that the optimal molecular size for maximum immunomodulatory activity is between 5 kDa and 400 kDa; polysaccharides smaller than 5 kDa or larger than 400 kDa exhibited only marginal immunomodulatory activity (Im et al., 2005). In this study, we examined the *in vivo* immunomodulatory activity of orally administered cellulase-treated *Aloe vera* gel, PAG. The total content of polysaccharides with molecular weights higher than 3.5

kDa was 23.85% ± 0.35%, and the total content of the polysaccharides between 5 kDa and 400 kDa was 20.47%, as determined by HPLC analysis. Because fresh *Aloe* gel contains about 10% polysaccharides by dry weight with an average MW of 2,000 kDa, the content and molecular length of the polysaccharides contained in PAG is quite different from those contained in native *Aloe vera* gel (Qui et al., 2000).

Aloe vera gel and the polysaccharides isolated from *Aloe vera* gel exert immunomodulatory activity *in vivo* as well as *in vitro* (Reynolds and Dweck, 1999), but oral immunomodulatory efficacy has not been demonstrated. Here, we examined the immunomodulatory activity of orally administered PAG in both normal and diabetic mouse models. The immunomodulatory activity of orally administered PAG was first examined in a *Candida albicans*-infection model based on previous studies showing that acemannan, the major polysaccharide present in *Aloe vera* gel, mediates its activity mainly through activation of macrophages (Karaca et al., 1995; Zhang and Tizard, 1996; Ramamoorthy et al., 1996; Djeraba and Quere, 2000) and the macrophages activated with acemannan exert enhanced phagocytic and candidicidal activities (Stuart et al., 1997). The kidney is particularly susceptible to *Candia albicans* infection, and renal failure is the major cause of death in systemic candida infection (Parker et al., 1976). Furthermore, there has been a resurgence of the incidence of candida infection since the onset of the AIDS epidemic (Bandar et al., 2006). Thus, we examined the immunomodulatory activity of PAG in mice that were immunosuppressed and infected with *Candida albicans*.

As an immunosuppression model, T1DM was induced by injecting low doses of streptozotocin for five consecutive days into C57BL/6 mice, and the effects of PAG administration were examined using the *Candia albicans*-infection model. We found that oral administration of PAG dose dependently and significantly reduced the fungal burden in the kidney as well as in the spleen. The number of CFUs in the spleen and kidney of untreated T1DM mice was much higher than normal mice, and the fungal reduction by PAG was weaker in T1DM mice than in normal mice. Streptozotocin exerts systemic toxic effects, and thus the streptozotocin-induced T1DM model may be not a good model to examine the immunomodulatory activity of PAG.

Acemannan exhibits adjuvant activity in vaccinations against virus (Chinnah et al., 1992) or heart worm antigens (Usinger, 1997), and increases survival rates in virus-infected animals (Sheets et al., 1991). Because acemannan does not exert anti-viral activity *per se*, it may activate the generation of CTLs specific to viral antigens. Thus, we examined whether oral PAG administration could enhance the generation of antigen-specific CTL activity in mice immunized with soluble OVA using *in vivo* CTL assays. Administration of PAG did not increase OVA-specific CTL activity in normal mice. However, administration of PAG dose dependently restored the suppressed CTL generating capacity in DIO mice, which resembles human T2DM in terms of metabolic abnormalities, such as hyperglycemia, obesity, and insulin resistance (Surwit et al., 1988; Mills et al., 1993; Wencel et al., 1995). Although the mechanisms underlying the *in vivo* immunomodulatory activity of PAG remain to be elucidated, this is the first report demonstrating the immunomodulatory activity of orally administered *Aloe vera* gel.

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REFERENCES

Bandar, I. N., Widodo, D., Djauzi, S., Muthalib, A., Soegondo, S., and Wahyuningsih, R., Correlation between CD4 count and intensity of Candida colonization in the oropharynx of HIV-infected/ AIDS patient. *Acta Med. Indones*., 38, 119-125 (2006).

Chinnah, A. D., Baig, M. A., Tizard, I. R., and Kemp, M. C., Antigen dependent adjuvant activity of a polydispersed beta-(1,4)-linked acetylated mannan (acemannan). *Vaccine,* 10, 551-557 (1992).

Djeraba, A. and Quere P., *In vivo* macrophage activation in chickens with Acemannan, a complex carbohydrate extracted from *Aloe vera*. *Int. J. Immunopharmacol.,* 22, 365-372 (2000).

Egger, S. F., Brown, G. S., Kelsey, L. S., Yates, K. M., Rosenberg, L. J., and Talmadge, J. E., Hematopoietic augmentation by a beta-(1,4)-linked mannan. *Cancer Immunol. Immunother.,* 43, 195-205 (1996).

Gowda, D. C., Neelisiddaiah, B., and Anjaneyalu, Y. V., Structural studies of polysaccharides from *Aloe vera*. *Carbohydr. Res.,* 72, 201-205 (1979).

Harris, C., Pierce, K., King, G., Yates, K. M., Hall, J., Tizard, I., Efficacy of acemannan in treatment of canine and feline spontaneous neoplasms. *Mol. Biother.,* 3, 207-213 (1991).

Im, S. A., Oh, S. T., Song, S., Kim, M. R., Kim, D.-S., Woo, S.

S., Jo, T. H., Park, Y. I., and Lee, C. K., Identification of optimal molecular size of modified Aloe polysaccharides with maximum immunomodulatory activity. *Int. Immunopharmacol.,* 5, 271-279 (2005).

Karaca, K., Sharma, J. M., and Nordgren, R., Nitric oxide production by chicken macrophages activated by Acemannan, a complex carbohydrate extracted from *Aloe vera*. *Int. J. Immunopharmacol.,* 17, 183-188 (1995).

King, G. K., Yates, K. M., Greenlee, P. G., Pierce, K. R., Ford, C. R., McAnalley, B. H., Tizard, I.R., The effect of Acemannan Immunostimulant in combination with surgery and radiation therapy on spontaneous canine and feline fibrosarcomas. *J. Am. Anim. Hosp. Assoc.,* 31, 439-447 (1995).

Lee, J. K., Lee, M. K., Yun, Y.-P., Kim, Y., Kim, J. S., Kim, Y. S., Kim, K., Han, S. S., and Lee, C. K., Acemannan purified from Aloe vera induces phenotypic and functional maturation of immature dendritic cells. *Int. Immunopharmacol.,* 1, 1275-1284 (2001).

Lee, Y. H., Lee, Y.-R., Im, S. A., Park, S. I., Kim, K. H., Gerelchuluum, T., Song, S., Kim, K., and Lee, C. K., Calcineurin inhibitors block MHC-restricted antigen presentation *in vivo*. *J. Immunol.,* 179, 5711-5716 (2007).

Mandal, G. and Das, A., Structure of the glucomannan isolated from the leaves of *Aloe barbadensis* Miller. *Carbohydr. Res.,* 87, 249-256 (1980).

Manna, S. and McAnalley, B. H., Determination of the position of the O acetyl group in β-(1-4) mannan (acemannan) from *Aloe barbardensis Miller*. *Carbohydr. Res.,* 241, 317-319 (1993).

Mills, E., Kuhn, C. M., Feinglos, M. N., and Surwit, R., Hypertension in CB57BL/6J mouse model of non-insulin-dependent diabetes mellitus. *Am. J. Physiol.,* 264, R73-R78 (1993).

Parker, J. C., Jr., McCloskey, J. J., Solanki, K. V., and Goodman, N. L., Candidosis: the most common postmortem cerebral mycosis in an endemic fungal area. *Surg. Neurol.,* 6, 123-128 (1976).

Peng, S. Y., Norman, J., Curtin, G., Corrier, D., McDaniel, H. R., and Busbee, D., Decreased mortality of Norman murine sarcoma in mice treated with the immunomodulator, Acemannan. *Mol. Biother.,* 3, 79-87 (1991).

Qiu, Z., Jones, K., Wylie, M., Jia, Q., and Orndorff, S., Modified Aloe barbadensis polysaccharide with immunoregulatory activity. *Planta Med.*, 66, 152-156 (2000).

Ramamoorthy, L., Kemp, M. C., and Tizard, I. R., Acemannan, a beta-(1,4)-acetylated mannan, induces nitric oxide production in macrophage cell line RAW 264.7. *Mol. Pharmacol.,* 50, 878-884 (1996).

Reynolds, T. and Dweck, A. C., *Aloe vera* gel leaf: a review update. *J. Ethnopharmacol.,* 68, 3-37 (1999).

Sheets, M. A., Unger, B. A., Giggleman, G. F., Jr., and Tizard, I. R., Studies of the effect of acemannan on retrovirus infections: clinical stabilization of feline leukemia virus-infected cats. *Mol. Biother.*, 3, 41-45 (1991).

Stuart, R. W., Lefkowitz, D. L., Lincoln, J. A., Howard, K., Gelderman, M. P., and Lefkowitz, S. S., Upregulation of phagocytosis and candidicidal activity of macrophages exposed to the immunostimulant acemannan. *Int. J. Immunopharmacol.,* 19, 75-82 (1997).

Surwit, R. S., Kuhn, C. M., Cochrane, C., McCubbin, J. A., and Feinglos, M. N., Diet-induced type II diabetes in C57BL/6J mice. *Diabetes,* 37, 1163-1167 (1988).

Usinger, W. R., A comparison of antibody responses to veterinary vaccine antigens potentiated by different adjuvants. *Vaccine,* 15, 1902-1907 (1997).

Wencel, H. E., Smothers, C., Opara, E. C., Kuhn, C. M., Feinglos, M. N., and Surwit, R. S., Impaired second phase insulin response of diabetes-prone C57BL/6J mouse islets. *Physiol. Behav.*, 57, 1215-1220 (1995).

Yagi, A., Makino, K., Nishioka, I., and Kuchino, Y., Aloe mannan, ploysaccharide, from *Aloe arborescens var. natalensis*. *Planta Med.,* 31, 17-20 (1997).

Yates, K. M., Rosenberg, L. J., Harris, C. K., Bronstad, D. C., King, G. K., Biehle, G. A., Walker, B., Ford, C. R., Hall, J. E., and Tizard, I. R., Pilot study of the effect of acemannan in cats infected with feline immunodeficiency virus. *Vet. Immunol. Immunopathol.,* 35, 177-189 (1992).

Zhang, L. and Tizard, I. R., Activation of a mouse macrophage cell line by acemannan: the major carbohydrate fraction from *Aloe vera* gel. *Immunopharmacol.,* 35, 119-128 (1996).