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Mangifera indica L. (Anacardiaceae) Stem Bark Extract Inhibits Mice Humoral Immune Response. Preliminary Results

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SUMMARY. The effect of the ethanolic extract of stem bark of *Mangifera indica* (MSE) on the primary immune response was studied in mice injected with sheep red blood cells (SRBC), a T-dependent antigen. This extract showed a suppressor effect on the production of IgM and IgG anti-SRBC. The extract at a dose of 20mg/kg ip caused a slight suppression effect reducing significantly the IgG levels by 29.5 and 22.8% on the seventh and tenth days, respectively. However, the dose of 80 mg/kg ip was more suppressive decreasing significantly the IgM (42.3%) and IgG levels (75.7%) on the fourth day. The suppression of the IgG level was maintained during primary immune response. It was more intense on the seventh (58.6%) and tenth days (45.0%) and decreased (37.4%) on the twenty-first day. In the spleen, the higher dose also caused a reduction (45.1%) of the number of IgM secreting cells on the fourth day, and of IgM (63.1%) and IgG (53.7%) secreting cells on the ninth day. The dose of 80 mg/kg of MSE injected ip 1 h before SRBC increased the percentage of phagocytic cells in the peritoneal cavity of mice. The results show that MSE reduces the IgM and IgG anti-SRBC levels, which may be associated with a reduction of Ig-forming cells and increased migration of phagocytes into the peritoneal cavity.

RESUMEN. "El extracto de corteza de tallo de *Mangifera indica* L. (Anacardiaceae) inhibe la respuesta inmune humoral en ratones. Resultados Preliminares". El efecto del extracto etanólico de corteza de tallo de *Mangifera indica* (MSE) sobre la respuesta inmune ha sido estudiado en ratones inyectados con glóbulos rojos de oveja (SRBC), un antígeno timo-dependiente. El extracto en una dosis de 80 mg/kg por vía intraperitoneal mostró un efecto supresor sobre la producción de IgM (42,3%) e IgG (75,7%) anti-SRBC en el cuarto día. La supresión del nivel de IgG ha sido mantenida durante la respuesta inmune. Esta dosis también causó una reducción del número de células secretoras de IgM (el 63,1%) y IgG (el 53,7%) en el bazo y aumentó el porcentaje de células fagocíticas en la cavidad peritoneal de los ratones. Los resultados muestran que MSE reduce los niveles de IgM y IgG que pueden ser asociados con una reducción de células formadoras de inmunoglobulinas en el bazo y migración aumentada de fagocitos en la cavidad peritoneal.

INTRODUCTION

The immunomodulating effects of substances extracted from plants have been demonstrated in animals [1,2] as well as in humans [3,4]. However, information on the effects of Brazilian plants on the humoral and cellular responses are scarce [5,6].

Leaves, stem bark, seeds and fruits of *M. indica* L., popularly known as the mango tree, have been reported to have therapeutic properties. The stem bark is used for the treatment of fever, leucorrhoea, diarrhoea *inter alia*. The leaves are used in the treatment of asthma, cough, diarrhoea [7] and diabetes [8].

In vitro assays have demonstrated that the ethanolic stem bark extract of *M. indica* has antiamoebic activity [9] and that the leaf extract has inhibitory activity against *M. tuberculosis* [10] and

KEYWORDS: Antibody production; IgM and IgG anti-SRBC; *Mangifera indica* L. (Anacardiaceae); Phagocytosis; Plaque-forming cells; Stem bark extract.
PALABRAS CLAVE: Células formadoras de placas; Extracto etanólico de corteza de tallo; Fagocitosis; IgM y IgG Anti-SRBC; *Mangifera indica* L. (Anacardiaceae); Producción de anticuerpos.

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herpes simplex virus [11]. The anti-herpes virus type 2 activity in the leaf extract is associated with mangiferin, a tetrahydroxy pyrrolidone saponin [11,12].

Different effector functions induced during immune response can be influenced by the type and concentration of antigen [13,14]. SRBC (sheep red blood cells) is a T-dependent antigen, which induces humoral or cellular responses when administered in high or low doses, respectively [15,16].

The present study evaluates the effect of the ethanolic extract of *M. indica* stem bark (MSE) on the mice humoral immune response against SRBC.

MATERIALS AND METHODS

Animals

Eight to ten weeks old Swiss female mice obtained from the animal house of the Laboratório de Tecnologia Farmacêutica, Universidade Federal da Paraíba (UFPB) were used. They were kept in an air conditioned room (25 ± 1,0 °C) under a 12 h light - dark cycle and maintained with food and water *ad libitum*.

Preparation of the ethanolic extract

The stem bark of *M. indica* trees was harvested from localities around the UFPB and the bark was dried in an oven at 35-40 °C. These samples were pulverized (325 g) and the extract was obtained by Soxhlet extraction with 92 % ethanol in water at 70 °C for 4 days. The MSE obtained was concentrated in a rotary evaporator, delipidated, pulverized (25 g) and stored at 4 °C.

Acute toxicity test

Fasting (15 h) male and female mice were inoculated ip with 37.5-1000 mg/kg of MSE, in a dose-volume of 10 ml/kg. The animals were observed for lethality during 72 h and the LD50 value was determined graphically from probit-log dose curve.

Preparation of antigen

The SRBC antigen in Alsever 's solution was washed with PBS and 1.6 x 10^8 cells in 0.5 ml of PBS were injected ip for animal.

Induction and quantification of IgM and IgG anti-SRBC

Groups of ten mice were injected with PBS or MSE 20 or 80 mg/kg ip. Twenty-four hours later, a SRBC suspension was also administered ip into these animals. The mice were bled by retro-orbital puncture on days 4, 7, 10 and 21 after SRBC administration. The sera obtained were inactivated (56 °C, 40 min) and assayed for hemagglutinin (HA) titre [17]. Briefly, the HA titre of IgM was determined by incubation of SRBC with the diluted sera (up to a 1:4096) and the IgG, by the same process, with addition of 0.1 M 2-mercaptoethanol (Merck) at 37 °C, for 30 min. The results of 3 such experiments were expressed as the mean of the HA titre ($\log_2$) ± standard error (SE).

Detection of antibody-producing cells

The number of hemolytic plaque forming cells was determined by the method of Dresser [18]. Groups of ten mice were administered with PBS or MSE 20 or 80 mg/kg ip, followed 24 h later by the inoculation of SRBC ip. Four and nine days after the antigenic stimulation, the spleens were collected in Hank's saline. The spleens were homogenized and aliquots of the cell suspension were mixed with 5% SRBC and 0.8% agarose (Merck) for direct plaques and further addition of rabbit anti-mouse IgG sera for indirect plaques. The cell suspensions were incubated on glass slides for 2h, at 37 °C. Guinea pig serum, previously absorbed and diluted was added and incubated for 1h. The slides were fixed in 4% paraformaldehyde. The number of indirect plaques was determined by subtracting the number of direct plaques from the total plaques developed with anti-IgG. The results of 3 experiments were expressed as means of the number of direct and indirect plaques / 10^6 cells ± SE.

In vivo Phagocytosis of SRBC

In vivo phagocytic activity was analyzed by determining the percent of peritoneal cells with ingested SRBC as described by Rocha [19]. Groups of ten mice injected 24 h earlier with PBS or MSE 20 or 80 mg/kg ip were inoculated with SRBC. After 30 or 60 min the animals were sacrificed and their peritoneal cavities washed with Hank's saline. The cells were collected and counted. Aliquots of peritoneal cells (1 x 10^6 cells/ml) were allowed to attach to glass microslides for 30 min at 37 °C. The microslides were washed, fixed in methanol-acetone for 1-2 min and stained with Giemsa. The percent of macrophages containing SRBC was determined by random microscopical examination of at least 200 cells. The results were calculated as the percent of cells that phagocytized at least 3 SRBC in

relation to the total number of cells. The average number of intracellular SRBC per phagocyte was also determined.

RESULTS

Toxicity

The LD_{50} value, obtained by graphic interpolation was 912.01 mg/kg ip.

Anti-SRBC serum IgM and IgG concentrations

The effects of MSE on the primary humoral immune response to SRBC are shown in Figure 1A and 1B. The dose of 20 mg/kg, which did not alter the IgM levels, significantly reduced IgG levels by 29.5 and 22.8%, at the 7th and the 10th day, respectively. The effect of 80 mg/kg was evident on the 4th day as well when the levels of both IgM and IgG were reduced by 42.3 and 75.7%, respectively. While the IgM levels were maintained similar to the control values during the rest of the period, the higher dose of MSE significantly decreased the IgG levels by: 58.6, 45.0 and 37.4% during the 7th, 10th and 21st days, respectively. Thus, MSE injected ip, 24 h before the SRBC, caused a dose-dependent suppression in both IgM and IgG anti-SRBC titres, and the effect was greater and more persistent in the case of IgG levels.

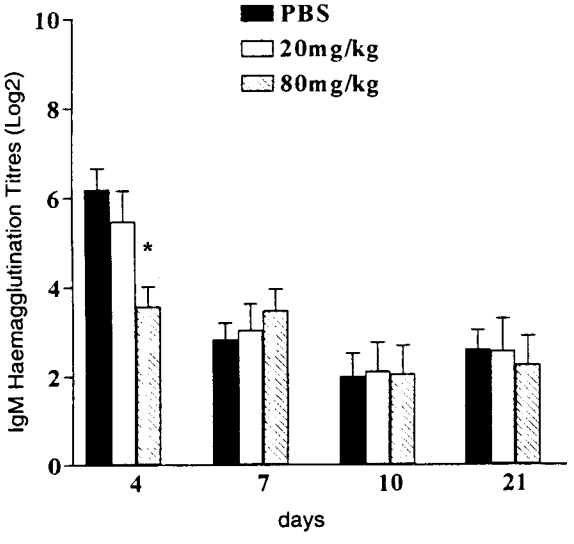

Figure 1A. Effect *in vivo* of the MSE on primary immune response to SRBC. The histogram shows the IgM titer in mice treated with PBS (■), MSE 20 (❑) and 80 mg/kg (▨) and inoculated, 24 h after, with SRBC. The results are expressed in log 2 of haemagglutinin and represent the mean ± SE (14-16 animals per group). * $p< 0.05$.

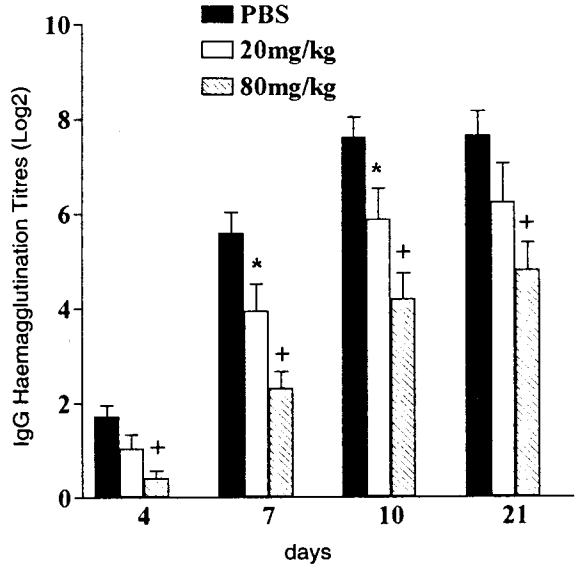

Figure 1B. Effect *in vivo* of MSE on the primary immune response to SRBC. The histogram shows the IgG titre in mice treated with PBS (■), MSE 20 (❑) and 80 mg/kg (▨) and inoculated, 24 h after, with SRBC. The results are expressed in log 2 of haemagglutinin and represent the mean ± SE (14-16 animals for group). * $p< 0.05$ + $p< 0.01$.

Spleen cells producing anti-SRBC immunoglobulins

While the animals injected with MSE 20 mg/kg did not show alterations, the dose of 80 mg/kg caused a reduction of 45.1% in the number of splenic IgM-producing cells on the 4th day (Figure 2). This same dose also caused a reduction of 63.1% of IgM and 53.7% of IgG secreting cells on the 9th day (Figure 2). However, the number of IgM secreting cells was very much reduced in the control animals on the 9th day when compared with their number on the 4th day. Although the number of antibody-producing cells had been reduced in the mice, there was no change in the number of the total and viable splenic cells or in the splenic index (data not shown).

Phagocytic activity by peritoneal cells

In the animals injected with 20 mg/kg MSE, 24 h before the SRBC, there was no change in the percent of phagocytic cells (Figure 3) or in the number of SRBC ingested by these cells in the peritoneal cavity (data not shown). However, the dose of 80 mg/kg significantly increased the percent of phagocytic cells by 62.5%, 60 min

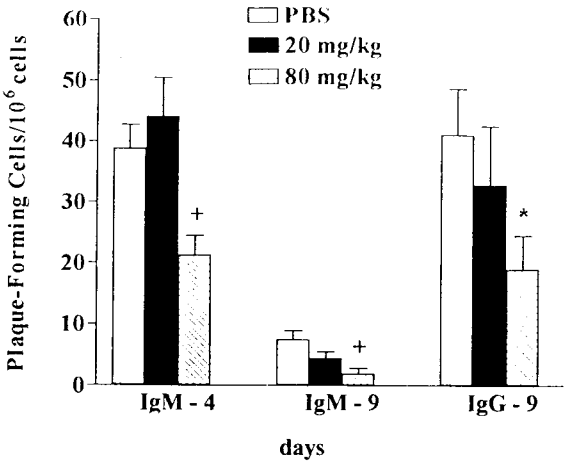

Figure 2. Effects *in vivo* of MSE on IgM and IgG -producing cells. The mice had treated ip with PBS, 20 and 80 mg/kg of MSE 24 h before i.p. SRBC injection. The spleens were collected in the fourth or ninth day and the direct (IgM) and indirect (IgG) plaque-forming cells were determined. The results are expressed as the mean ± SE of groups of 7-12 animals.
* $p< 0.05$ + $p< 0.01$.

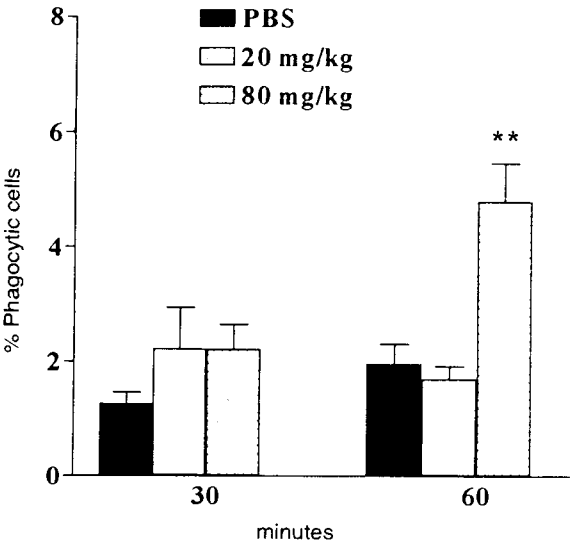

Figure 3. Effect *in vivo* of MSE on phagocytosis of SRBC. The histogram shows the percentage of phagocytic cells when the mice were treated ip with PBS, 20 and 80 mg/kg of MSE, 24 h before ip injection of SRBC. The peritoneal cells were collected at 30 or 60 min after SRBC administration. The results are expressed as the means ± SE of groups of 10-16 animals. ** $p< 0.001$

after the SRBC inoculation. No alteration was found in the number of SRBC ingested per cell during this period (data not shown). However, the cell morphology showed an increased level of activity, as evident from the presence of a large number of vacuoles and an increase in cell size.

DISCUSSION AND CONCLUSIONS

The humoral immune response against a T-dependent antigen, such as SRBC, is initiated with the recognition of the antigen by antigen-presenting cells (APCs), for example, macrophages, dendritic cells and B lymphocytes [20-22]. The majority of the resting B lymphocytes in the peripheral circulation produce both membrane IgM and IgD [23,24] and when they are activated by antigen and T lymphocytes, produce an increasing proportion of these immunoglobulins in a secreted form [25,26]. Some of these activated B lymphocytes undergo heavy chain class switching and begin to express and secrete other antibodies such as IgG, IgA and IgE [27-30]. The regulation of the antibody production and the heavy chain switching are dependent on the expression of costimulatory molecules and cytokine receptors on the B lymphocytes [31-33], the quantity and the ratio of cytokines secreted by APCs and by Th1 and Th2 subpopulations [34-36].

The present data indicate that in mice, MSE caused a dose-dependent reduction of IgM and IgG antibodies during the primary immune response induced by SRBC. The results also showed that the IgG rather than the IgM concentration level was more suppressed.

The classic immunosuppressive agents such as purine and pyrimidine analogs, folic acid antagonists and X-rays have similar effects in reducing the IgG more than that of IgM responses by interfering at the S phase in the B cell reproduction cycle [37,38]. Studies with LPS-activated B cells suggest that immunoglobulin isotype switching occurs during DNA synthesis at the S phase [39]. Furthermore, switching to IgG might involve an asymmetric cell division, in which one of the daughter cells secretes IgG, while the other secretes IgM [40].

Thus MSE may also have a direct or indirect suppressor effect on B lymphocyte by reducing the number of IgM secreting cells and a consequent decrease in the number of these cells, which proliferate and give origin to the IgG secreting cells. This hypothesis is supported by the observed reduction (45.1%) of IgM secreting cells on the 4th day, with the dose of 80 mg/kg, when the majority of these cells had not suffered heavy chain switching. Further, there also occurred a reduction of 53.7% of the IgG secreting cells, on the 9th day, when the levels of this immunoglobulin begin to increase in relation to the IgM levels.

The possibility that the reduction of Ig secreting cells was caused by a toxic effect of MSE on B lymphocytes is less likely as the higher dose utilized was approximately ten times less than the acute LD_{50} dose. The absence of alterations in the number, and the viability of the splenic cells and the splenic index (data not shown) suggest that the action of MSE in reducing the B cell population may have been masked by a corresponding increase in other cells. Preliminary results have demonstrated that if MSE is administered seven days after SRBC the extract did not present suppressor effect. These results support the hypothesis that MSE did not have any B lymphocyte toxicity.

Initial studies demonstrated that 10^5 SRBC inoculated intravenously in mice induces Delayed Type Hypersensitivity (DTH) while 10^8 SRBC induces high hemagglutinin concentrations [16]. The immune response against low and high number of SRBC correlates with Th1 or Th2 patterns of cytokine production, respectively [41]. In this paper, the SRBC number inoculated probably induced a humoral immune response (Th2 pattern). Whether the reduction of the IgG-secreting cells and the IgG levels were caused by the direct suppression of Th2 lymphocytes or the induction of cytokine production by APCs is not known. Preliminary studies demonstrated that the maximum suppressor effect of MSE extract was obtained when it is inoculated three days after SRBC inoculation. In this phase of the immune response, IgM secreting B lymphocytes are activated by T lymphocytes and under actions of cytokines proliferate and switch the Immunoglobulin class [42,43].

Thus, studies on the functions of phagocytic cells show that the dose of 80 mg/kg caused an increase in the percentage of these cells in the peritoneal cavity. This effect can be mediated by tannins presents in MSE since these substances have chemotactic activity for alveolar macrophages [44]. Therefore the reduction of antibody secretion may not been caused by an inhibition of the cell migration into the site of antigen inoculation.

Further the MSE did not inhibit or increase the phagocytic process, demonstrating that the extract does not modify the recognition and ingestion of antigen by phagocytic cells. However, changes in the morphology of the phagocytic cells treated with MSE suggest a greater degree of activation of these cells, which can modify the process of digestion and presentation of antigen. For example, studies have demonstrated that hyperactivated cells produce lisosomic exo- and endopeptidases, which digest proteins into amino acids and very small oligopeptides, with reduction of immunogenicity and production of antibodies [45]. In addition, hyperactivity of phagocytic cells results in a greater release of mediators such as hydrogen peroxide, nitric oxide (NO) and prostaglandins, which may suppress the lymphocyte activity [47,48].

A direct effect of tannins present in the extract may contribute to the inhibitory effect of MSE on antibody production as tannins have strong affinity for proteins and this effect may alter the functions of different cells of the immune system [48,49]. Further, tannic acid increases covalent binding [50] and thus stimulates the phagocytic process. Macrophage activation and IL-1β production have been associated with anti-tumoral activity of tannins [51,52].

In summary, the results indicate clearly that MSE has an inhibitory effect on humoral immune response. However, further chemical and immunological studies are needed to isolate the active principles and to clarify the mechanisms of action of MSE.

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REFERENCES

1. Kim, H.M., M.J. Kim, E. Li, Y.S. Lyu, C.Y. Hwang & N.H. An (1999) *J. Ethnopharmacol.* **67**: 163-9
2. Agarwal, R., P. Diwanay & B. Patki (1999) *J. Ethnopharmacol.* **67**: 27-35
3. Seow, W.K., A. Ferrante, L.S. Ying & Y.H. Thong (1989) *Clin. Exp. Immunol.* **75**: 47-51
4. Mazor, R.L., I.Y. Menendez, M.A. Ryan, M.A. Fiedler & H.R. Wong (2000) *Cytokine* **12**: 239-45
5. Rossi-Bergman, B., S.S. Costa & V.L.G. Moraes (1997) Ciência e Cultura *J. Braz. Assoc. Adv. Science* **49**: 395-401
6. Piuvezam, M.R., L.M.T. Peçanha, J. Alexander & G. Thomas (1999) *J. Ethnopharmacol.* **67**: 93-101
7. Camargo, M.T.L.A. (1988) "Plantas medicinais e de rituais afro-brasileiros" en "Plantas Medicinais de Rituais Afro-brasileiros" (C.L.R. Balieiro, ed.), Almed, São Paulo, Brasil, págs. 46-8

8. Aderibigbe, A.O., T.S. Emudianughe & B.A. Lawal (1999) *Phytother. Res.* **13**: 504-07
9. Tona, L., K. Kambu, N. Ngimbi, K. Cimanga & A.J. Vlietinck (1998) *J. Ethnopharmacol.* **61**: 57-67
10. Frame, A.D., E. Rios-Olivares, L. De Jesus, D. Ortiz, J. Pagan & S. Mendez (1998) *P.R. Health Science J.* **17**: 243-52
11. Yoosook, C., N. Bunyapraphatsara, Y. Boonyakiat & C. Kantasuk (2000) *Phytomedicine* **6**: 411-9
12. Zhu, X.M., J.X. Song, Z.Z. Huang, Y.M. Wu & M.J. Yu (1993) *Chuang Kuo Yao Li Hsueh Pao* **14**: 452-4
13. Bretsher, P.A., G. Wei, J.N. Menon & H.B. Ohmann (1992) *Science* **257**: 539-42
14. Bancroft, A.J., K.J. Else & R.K. Grencis (1994) *Eur. J. Immunol.* **24**: 3113-18
15. Lagrange, P.H., G.B. Mackaness & T.E. Miller (1974) *J. Exp. Med.* **139**: 528-41
16. Mackaness, G.B., P.H. Lagrange, T.E. Miller & T. Ishibashi (1974) *J. Exp. Med.* **139**: 543-59
17. Hudson, L. & F.C. Hay (1989) "Antibody interaction with antigen", en "Practical Immunology" (Elaine and Francis, eds.), Blackwell Sci. Publications, Oxford, págs. 251-4
18. Dresser, D.W. (1978) "Assays for Immunoglobulin-secreting cells", en "Handbook of Experimental Immunology" (D.M.Weir, ed.), Blackwell Sci.Publications, Oxford, págs. 8.1-9
19. Rocha, N.P. (1989) *Braz. J. Méd. Biol. Res.* **22**: 1401-3
20. Benacerraf, B. (1978) *J. Immunol.* **120**: 1809-12
21. Ashwell, J.D. (1988) *J. Immunol.* **140**: 3697-700
22. Germain, R.N. & D.H. Margulies (1993) *Annu. Rev. Immunol.* **11**: 403-50
23. Havran, W.L., D.L. DiGiusto & J.C. Cambier (1984) *J. Immunol.* **132**: 1712-6
24. Alés-Martinez, J.E., E. Cuende, C. Martinez-A, R.M.E. Parkhouse, L. Pezzi & D.W. Scott (1991) *Immunol. Today* **12**: 201-5
25. Hodgkin, P.D., L.C. Yamashita, B. Seymour, R.L. Coffman & M.R. Kehry (1991) *J. Immunol.* **147**: 3696-702
26. Parker, D.C. (1993) *Annu. Rev. Immunol.* **11**: 331-60
27. Del Prete, G.F., M. De Carli, M. Ricci & S. Romagnani (1991) *J. Exp. Med.* **174**: 809-13
28. Schultz, C.L., P. Rothman, R. Kühn, M. Kehry, W. Müller, K. Rajewsky & R.L. Coffman (1992) *J. Immunol.* **149**: 60-4
29. Harriman, W., H. Völk, N. Defranoux & M. Wabl (1993) *Annu. Rev. Immunol.* **11**: 361-84
30. Snapper, C.M. & F.D. Finkelman (1999) "Immunoglobulin Class Switching", en "Fundamental Immunology" (W. E. Paul ed.), Lippincott-Raven Publishers, Philadelphia, págs. 831-61
31. Freedman, A.S., G.J. Freeman, K. Rhynhart & L.M. Nadler (1991) *Cell. Immunol.* **137**: 429-37
32. Stack, R.M., D.J. Lenschow, G.S. Gray, J.A. Bluestone & F.W. Fitch (1994) *J. Immunol.* **152**: 5723-33
33. Lenschow, D.J., T.L. Walunas & J.A. Bluestone (1996) *Annu. Rev. Immunol.* **14**: 233-58
34. Seder, R.A., W.E. Paul, M.M. Davis & B. Fazekas de St Groth (1992) *J. Exp. Med.* **176**: 1091-8
35. Scott, P. (1993) *Science* **260**: 496-7
36. Seder, R.A. & W.E. Paul (1994) *Annu. Rev. Immunol.* **12**: 635-73
37. Barrett, J.T. (1983) "Biologic aspects of the immune response" en "Textbook of Immunology - An Introduction to Immunochemistry and Immunobiology" (S.E. Harshberger, ed.), The C.V. Mosby Company, St. Louis, págs.157-62
38. Winkelstein, A. (1997) "Immunossupressor Therapy" en "Medical Immunology" (D.P.Stites, A.I.Terr and T.G.Parslow, eds.), Appleton & Lange, págs. 634-48
39. Kenter, A.L. & J.V. Watson (1987) *J. Immunol. Methods* **97**: 111-7
40. van der Loo, W., E. Severinson-Gronowicz, S. Strober & L.A. Herzenberg (1979) *J. Immunol.* **122**: 1203-8
41. Li, L., J.F. Elliott & T.R. Mosmann (1994) *J. Immunol.* **153**: 3367-78
42. Siekevitz, M., C. Kochs, K. Rajewisky & R. Dildrop (1987) *Cell* **48**: 757-70
43. MacLennan, I.C., Y.J. Liu & G.D. Johnson (1992) *Immunol. Rev.* **126**: 143-61
44. Rohrbach, M.S., T. Kreofsky, R.A. Rostald & J.A. Russel (1989) *Am. Rev. Respir. Dis.* **139**: 39-45
45. Chain, B.M., M. Londei & M. Feldmann (1986) *Ann. Inst. Pasteur/Immunol.* **137D**: 320-3
46. Metzger, Z., S.T. Hoffeld & J.J.Oppenheim (1980) *J. Immunol.* **124**: 983-8
47. Deguchi, M., K. Inaba & S. Muramatsu (1995) *Immunol. Lett.* **45**: 157-62
48. Costa, A.F. (1977) "Fármacos com taninos" en "Farmacognosia", Fundação Calouste Gulbenkian, Lisboa, Vol.1, págs. 906-59
49. Dawra, R.K., H.P.S. Makkar & B. Singh (1988) *Anal. Biochem.* **170**: 50-3
50. Rabinovitch, M. (1970) "Phagocytic recognition" en "Mononuclear Phagocytes" (R. van Furth, ed.), Blackwell Sci. Publications, Oxford, págs. 299
51. Miyamoto, K., M. Nomura, M. Sasakura, E. Matsui, R. Koshiura, T. Murayama, T. Furukawa, T. Hatano, T. Yoshida & T. Okuda (1993a) *Japan. J. Cancer Res.* **84**: 99-103
52. Miyamoto, K., T. Murayama, M. Nomura, T. Hatano, T. Yoshida, T. Furukawa, R. Koshiura & T. Okuda (1993b) *Anticancer Res.* **13**: 37- 42