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Carrageenan: a review of its effects on the immune system

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Abstract

Carrageenans (kappa, lambda and iota) are sulphated polysaccharides isolated from marine algae that can markedly suppress immune responses both in vivo and in vitro. Impairment of complement activity and humoral responses to T-dependent antigens, depression of cell-mediated immunity, prolongation of graft survival and potentiation of tumour growth by carrageenans have been reported.

The mechanism responsible for carrageenan-induced immune suppression is believed to be its selective cytopathic effect on macrophages. This property of carrageenan has led to its adoption as a tool for analysing the role of these cells in the induction and expression of immune reactivity. Systemic administration of carrageenan may, however, induce disseminated intravascular coagulation and inflict damage on both the liver and kidney. This is an important consideration in the interpretation of the effects of carrageenan in vivo and precludes its use as a clinical immune suppressant.

Introduction

Carrageenan has traditionally been used to induce experimental inflammation in laboratory animals, and carrageenan-induced footpad oedema has become established as the most popular model for evaluation of prospective anti-inflammatory drugs. A comprehensive review of the biological effects of carrageenan was conducted by DI ROSA [23], but since this survey of the literature in 1972 several authors have reported the capacity of carrageenan, when injected subcutaneously or systemically, to markedly affect immune responses. These findings extend not only to effects on macrophages, towards which carrageenan may be selectively cytotoxic, but also to interference with responses such as antibody production, allograft rejection and delayed-type hypersensitivity reactions which are mediated by lymphocytes. The purpose of this paper is to briefly review the effects of carrageenan on the immune system; to discuss the mechanism of carrageenan-induced immune suppression; to appraise the use of carrageenan as a probe in the analysis of immune reactivity and to outline the pathological consequences of systemic carrageenan administration which at present preclude its use as an immunotherapeutic agent.

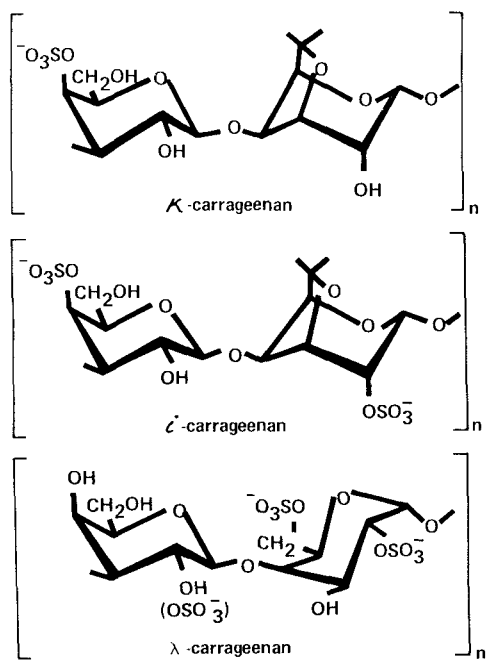

Figure 1
The structural formulas of kappa, iota and lambda carrageenans.

1. Source and structure of carrageenans

Carrageenans are a heterogeneous mixture of sulphated polygalactans extracted from cell walls of certain algae of the Rhodophyta. The main source is *Chondrus crispus*, although carrageenans have also been prepared from stages in the life cycle of *Gigartina*, *Hypnea*, *Furcellaria* and other species [36]. Several fractions can be identified (Fig. 1) which differ in total sulphate content and distribution, as well as molecular configuration [3, 53]. Kappa carrageenan is composed of carbohydrate chains of α-(1,3)-linked units of carrabiose, forming a linear, alternating sequence of D-galactose-4-sulphate and 3,6-anhydro-D-galactose units. Iota carrageenan is a kappa-like structure with an additional half sulphate ester residue. Lambda on the other hand, is of varying composition, but consists almost entirely of equal proportions of 1,3 linked and 1,4 linked D-galactose units. X-ray diffraction studies have revealed that these molecules have a double helical conformation with covalent inter-chain bonds, forming both a secondary and tertiary structure which makes carrageenan particularly resistant to enzymatic degradation. This latter property has important implications with regards the cytopathic effect of carrageenan on macrophages (see below).

2. The effect of carrageenan on immune responses

There is considerable evidence that systemic carrageenan administration causes suppression of immune reactivity. These effects are summarized in Table 1. The particular carrageenan types, either crude or purified, to which the principal immunological effects of carrageenan have been attributed are also indicated both in Table 1 and Table 3.

Various authors have reported suppression by carrageenan of antibody production against thymus-dependent antigens, such as sheep erythrocytes [4, 6, 57, 73, 74] and dinitrophenylated bovine serum γ globulin [32]. However, humoral responses to T lymphocyte independent antigens, such as trinitrophenylated DEAE-dextran and trinitrophenylated-Ficoll are unaffected [32, 79]. Carrageenan not only depresses, but also delays the peak of the primary antibody response, its effect (in the case of the response to sheep erythrocytes in the mouse) being dependent on dose, frequency of administration and on the temporal relationship between carrageenan and antigen injection [57, 73]. Suppression of antibody production after carrageenan administration has not invariably been reported. Indeed, TURNER and HIGGINBOTHAM [75] found that intraperitoneal (i.p.) carrageenan either suppressed or enhanced humoral responses to various antigens, again depending on the time and route of carrageenan injection relative to that of antigen. These workers also reported that animals injected with carrageenan together with sheep erythrocytes showed little or no response to secondary antigen challenge. Few other authors have examined the influence of carrageenan on secondary responses, but the latter observation does not contrast with that of ASCHHEIM and RAFFEL [4], who found the i.p. carrageenan had no inhibitory effect on secondary responses when given before the primary challenge.

In addition to interfering with antibody production, carrageenan impairs serum complement activity. Several authors, including BORSOS et al. [10], DAVIES [21, 22] and WARD and COCHRANE [78], have reported inhibition of early complement components, in particular C_1, by carrageenans in vivo and in vitro. Clearly, interference with complement activation may underlie the antagonistic effect of carrageenans on several

Table 1
Immunosuppressive properties of carrageenan.

Effect	Carrageenan	References
1. *Humoral immunity*		
Decreased responses to T-dependent antigens	Seakem 9®; 21®; kappa; lambda; unspecified	4, 6, 32, 57, 73
2. *Complement activity*		
Inhibition of early components (C1 and C4)	Seakem 21®	10, 21, 22
3. *Cell-mediated immunity*		
Suppressed delayed hypersensitivity reactions	Seakem 21®	7, 59, 61
Impaired cytotoxicity reactions	Seakem 9®; lambda; iota	16, 35, 48, 56, 82

'Seakem', 'Gelcarin' and 'Viscarin' are trade names of Marine Colloids, Inc., Springfield, New Jersey.
'Unspecified' refers to unpurified carrageenan salts from various sources.

immunological processes, such as phagocytosis, chemotaxis and immune cytolysis.

Impairment of cell-mediated immunity by carrageenan was first described in 1969 by SCHWARTZ and LESCOWITZ [61], who produced a transient suppression of delayed hypersensitivity (DH) reactions by giving carrageenan i.p. to tuberculin-sensitive guinea pigs at the time of skin testing. Later, BICE et al. [7] found that if given i.p. before immunization, carrageenan could depress the development of subsequent DH and, if given around the time of skin testing, could impair the expression of existing DH. Additional studies by BOROS and SCHWARTZ [9] described suppression by i.p. carrageenan of primary hypersensitivity granulomas forming around *Schistosoma mansoni* eggs in mouse lung, but no effect of carrageenan on secondary, enhanced egg granulomas in previously sensitized animals was demonstrated. These effects of carrageenan on DH reactions are reflected in its impairment of other cell-mediated responses in laboratory animals, such as graft rejection and anti-tumour resistance (see below). Carrageenan does not however affect graft versus host activity [71] – a finding which supports the view that it is not toxic to lymphocytes.

Recently, there have been reports of the influence of carrageenan on cell-mediated immune responses in vitro which are consistent with the foregoing observations in carrageenan-treated animals. These reports include the suppression of pig [48] and mouse [56] mixed leucocyte reactions, and impairment of cytotoxic T cell responses [82], antibody-dependent cell-mediated cytotoxicity [16] and natural killer cell activity [35]. Thus carrageenan interferes with a variety of cellular immune mechanisms. The extent to which this reflects solely the impairment of one particular cell type (the macrophage) is at present a matter of debate.

3. The mechanism of carrageenan-induced immune suppression

Effects on macrophages

Little attention has been paid to the exact mechanism(s) underlying carrageenan-induced immune suppression. Most attempts to do so focus on the effect of carrageenan on macrophages, or more precisely cells of the mononuclear phagocyte system (MPS). These cells play an essential role in the induction of humoral and cell-mediated immune responses, including the processing of antigen and its presentation to B cells, the generation of helper T cells and the secretion of a proliferative signal to B cells [45, 76]. It is through a cytopathic effect on macrophages that carrageenan appears to mediate its immunosuppressive effect.

Macrophages readily ingest carrageenan [17, 50, 69], in contrast to lymphocytes, which are not actively phagocytic and which lack a well-developed lysosomal complex. Uptake of carrageenan by cultured macrophages is followed, within 36 h, by liberation into the culture medium of hydrolytic enzymes [2], derived from secondary lysosomes, within which carrageenan can be identified [17, 50]. The release of these enzymes is consistent with ultrastructural observations of breaches in the membranes investing carrageenan-containing phagolysosomes [50] and with cell lysis due to escape of degradative enzymes into the cytosol [17, 50]. CATANZARO et al. [17] found that the cytotoxic effect of carrageenan in vitro could be at least partly prevented by methylprednisolone – a synthetic lysosome stabilizer, and concluded that the lysosome was the site of initial damage. They postulated either a direct toxic effect of carrageenan on lysosomal membranes, or osmotic rupture of the latter, due to persistence within secondary lysosomes of osmotically active undegraded carrageenan which, because of its unique secondary and tertiary structure, is particularly resistant to biochemical degradation by lysosomal glycosidases. Examination of tissue macrophages from carrageenan-injected mice by FOWLER and colleagues [26] revealed carrageenan within secondary lysosomes of Kupffer cells. These carrageenan-containing lysosomes were frequently ruptured. This finding, together with the observed degradation of nuclei and cytoplasmic structures (e.g. mitochondria) and the appearance of autophagic vacuoles within Kupffer cells, is consistent with a cytopathic effect of carrageenan on these tissue macrophages.

On the basis of the above studies it has been proposed that the immunosuppressive effects of carrageenan in vivo are a result of damage inflicted on cells of the MPS. Support for this view comes from observations that the ability of carrageenan-treated animals to remove particulate material (micro-organisms, colloidal carbon, aggregated albumin or sheep erythrocytes) from the blood is impaired [27, 42, 44]. This is

Table 2
Effect of intravenous or intraperitoneal carrageenan on the uptake of ^{51}Cr-labelled SRBC by mouse liver or spleen.

Treatment[a]	1 h after ^{51}Cr-SRBC		
	Liver	Spleen	Blood
Control	56.3 ± 2.8	2.3 ± 0.4	1.0 ± 0.1
Carrageenan i.p.	33.5 ± 6.8	11.4 ± 3.6	22.8 ± 8.4
Carrageenan i.v.	7.3 ± 2.7	15.4 ± 4.0	66.6 ± 7.2
	24 h after ^{51}Cr-SRBC		
Control	45.9 ± 2.1	2.0 ± 0.2	0.2 ± 0.1
Carrageenan i.p.	32.4 ± 3.9	20.8 ± 5.8	0.6 ± 0.1
Carrageenan i.v.	18.6 ± 8.0	31.8 ± 9.1	0.6 ± 0.1

[a] 2 mg 24 h before SRBC.
Values represent % injected dose (mean ± S.E.).

demonstrated in Table 2, which shows that systemic injection of mice with unpurified carrageenan reduces the uptake of intravenously administered sheep erythrocytes by the liver. This depression of Kupffer cell activity is associated with a reduction in the uptake of antigen by the liver, and its increased uptake by the spleen [27]. It is likely that carrageenan also affects the activity of splenic macrophages since RUMJANEK et al. [57] found that these cells from carrageenan-treated mice could suppress immune responses in vitro.

The MPS recovers from carrageenan-induced blockade within 1–3 days [19, 27, 37, 57], a rate of recovery which resembles that after other sulphated polysaccharides, such as dextran sulphate [63]. This temporary impairment of macrophage activity may underlie the increased susceptibility of carrageenan-treated mice to induction of immunological tolerance – a finding which has led LUKIĆ and co-workers [40, 41] to suggest that macrophages have a regulatory role in tolerance induction.

Several workers have challenged the assumption that carrageenan is cytotoxic for macrophages in vivo. ASCHHEIM and RAFFEL found that macrophages obtained from carrageenan-injected animals had increased phagocytic activity, and when laden with antigen could still stimulate antibody production in normal recipients [4]. They deduced that the carrageenan must affect cells other than macrophages and were supported by SCHWARTZ and CATANZARO [59], who, when investigating the effect of carrageenan on DH reactions, concluded that carrageenan probably did not suppress the reaction purely by its macrophage cytotoxicity. An added complication is the proposal of SAWICKI and CATANZARO [58] that although carrageenan is taken up by macrophages, it may be cytotoxic only to those within the peritoneum and liver and thus not damage those within lymphoid tissue or bone marrow.

Recent in vitro studies have cast further doubt on the presumed selective toxicity of carrageenan for macrophages. SIMON and JONES [62] were unable to demonstrate a toxic effect of carrageenan on cultured mouse peritoneal macrophages which had clearly ingested the material. It would thus appear that ingestion of the polysaccharide alone is not the sole cause of injury. In their work on natural killer cell activity, KIESSLING et al. [35] found that toxicity of carrageenan was not restricted to macrophages, and suggested that at high doses its effect on leucocytes may be non-selective. These apparently conflicting observations may reflect variability between carrageenan preparations, and consequently it would appear essential to evaluate the cytotoxic capacity of carrageenan prior to its application in immunological analysis. Unless restriction of toxicity towards cells of the MPS can be demonstrated, no valid deductions concerning the role of macrophages based on effects achieved with carrageenan in immune responses can be made.

Effects on lymphocytes

Reports on the behaviour of lymphocytes in the presence of carrageenan have been inconsistent, and its influence on these cells remains to be fully elucidated. Several workers have examined the ability of carrageenan-treated lymphocytes to respond to mitogens. Although BICE et al. [7] and WONG and HERSKOWITZ [79] found that carrageenan did not interfere with phytohaemagglutinin (PHA)-induced lymphocyte transformation, other workers have noted either depression [51, 57] or potentiation of this response [8, 31, 70]. It has been suggested by PAWELEC and BRONS [48], that these varied findings reflect the different carrageenan doses used. They showed a depression of the lymphocyte response to PHA at low concentrations of carrageenan, whereas at higher concentrations the response was unaffected or potentiated. More recently, SIMIĆ and co-workers [52, 65] have observed potentiation of the PHA response, and

have demonstrated that the effect of carrageenan is mediated through enhancement of the helper activities of macrophages in mitogen-induced responses of T cells. They have argued that the enhanced mitogenic responses of T lymphocytes in the presence of carrageenan is due to release of soluble factors from carrageenan-'activated' macrophages, which, they suggest, elaborate greater amounts of these factors. It had been previously suggested by NAKAMURA et al. [43] that the potentiation of T cell responses to PHA may be brought about by release of proteases from macrophages, whereas suppression of the response may be due to release from these cells of protease inhibitors.

Reports of lymphocyte responses to bacterial lipopolysaccharide (LPS) (a B lymphocyte mitogen) in the presence of carrageenan are also inconsistent. QUAN et al. [51] found carrageenan to be an effective stimulator of DNA synthesis in mouse B cells, whereas THOMSON et al. [70] could only demonstrate a mild mitogenic response in this species. Other authors [57] observed that spleen cells from carrageenan-treated mice gave inconsistent results when cultured with LPS, and JANEZIC and co-workers [33] found that the humoral response to LPS in carrageenan-treated rats was reduced to approximately 20% of that in control animals. Their conclusion was that LPS, although a thymus-independent antigen, was however macrophage dependent. FOWLER and THOMSON [29] found that carrageenan induced synthesis of IgM in the rat, and suggested that this response may have been promoted in part by a mitogenic capacity of carrageenan for rat B lymphocytes.

4. The influence of carrageenan on tumour growth

Although it has been known for some time that carrageenan can induce malignancy in laboratory animals [18], attention in the last few years has focussed on the ability of carrageenan to potentiate growth of experimental tumours, including chemically induced fibrosarcomas [34, 46, 80], an adenovirus type 12 tumour [39] and a spontaneously arising, transplantable tumour [68]. NELSON and NELSON [47] found that carrageenan could either inhibit or enhance tumour growth, depending on the dose of tumour cells injected, and PUGH-HUMPHREYS et al. [49] reported that carrageenan was actually toxic for certain ascites tumour cells and could thereby inhibit their growth. Observations that carrageenan can promote tumour growth lend support to the view [1] that macrophages may be the mediators of immunological surveillance against small numbers of malignant cells. However, in a preliminary study, we have not observed a marked effect of carrageenan on the incidence of macrophages within experimental tumours [67].

5. The effect of carrageenan on graft rejection

A summary of the reported effects of carrageenan on tissue and organ transplantation is shown in Table 3. Prolongation of skin graft survival by carrageenan in mice has been reported by RUMJANEK and BRENT [56], whilst others have shown that carrageenan reduces resistance to bone marrow transplants in irradiated animals [38]. Organ grafts also survive longer after treatment of recipients with carrageenan. Thus improved survival of allogeneic hearts and kid-

Table 3
Survival of allogeneic grafts in carrageenan-treated hosts.

	Host	Carrageenan	References
1. *Prolonged*			
skin	mouse	kappa[a]; Seakem 9®	54–56
bone marrow	mouse	Seakem 9®; kappa, lambda, iota	20, 38, 81
kidney	dog	Gelcarin®[b], lambda[b]	12, 14, 15
heart	rat	lambda	24
2. *Unaffected*			
heart	pig	unspecified[b]	13
kidney	man	lambda[b]	12
pancreatic islets	rat	lambda	44

[a] Superior to lambda or iota.
[b] Carrageenan in combined therapy.

neys in rats and dogs respectively has been achieved using carrageenan [12, 15, 24]. This degree of success prompted CALNE [12] to use carrageenan together with conventional immunosuppressive drugs in prevention of transplant rejection in man. However, the pathological consequences of its systemic administration (see below) precluded its adoption as a clinical immunotherapeutic agent.

6. Pathological effects of systemic carrageenan injection and their alleviation

The pathology of carrageenan-induced inflammation has been the subject of considerable study and has been adequately reviewed [64]. However, the pathological consequences of systemic carrageenan administration have received attention only recently, and have been of paramount importance in the assessment of carrageenan as a clinical immune suppressant.

A striking observation following i.v. or i.p. injection of certain carrageenan preparations into experimental animals has been the appearance of ischaemic lesions at body extremities. This 'acronecrosis', which is regarded as a manifestation of disseminated intravascular coagulation (DIC), has been described in the ears and tails of mice, rats and dogs by several workers [15, 72, 73]. WALL, CALNE and WILKINS [77] attempted to alleviate this toxic effect of carrageenan by concomitant administration of heparin, but found that this drug offered no protection and indeed gave poorer immunosuppression. FOWLER et al. [30] however, found that alleviation of acronecrosis could be achieved by injection of the antiprotease aprotinin and, in addition, that this measure had either no effect on carrageenan-induced immune suppression or even enhanced the degree of suppression [28].

It has been reported that carrageenan is hepatotoxic [6, 26, 66]. Hepatocyte necrosis, infiltration of inflammatory cells and extramedullary haemopoiesis have been found in livers of carrageenan-treated mice [30]. Increases in serum transaminase activity [30] and in circulating levels of acute phase reactants, such as α_2-macroglobulin in the rat [29] provide further evidence for carrageenan-induced damage to liver parenchymal cells. Two mechanisms may underlie this hepatotoxicity. First, individual hepatocyte necrosis may be an indirect consequence of the cytotoxic effect of carrageenan on Kupffer cells, which would result in release of lysosomal enzymes with consequent damage to adjacent hepatocytes. A similar mechanism was described by FERLUGA and ALLISON [25] to explain the hepatitis which developed after injection of endotoxin in *C. parvum*-primed mice. It is therefore noteworthy that carrageenan increases susceptibility of animals to endotoxin [5]. Second, by activating Hageman factor [60], carrageenan may stimulate the vasoactive polypeptide system, promoting blood coagulation and inducing liver cell necrosis by compromising the blood supply to the tissue. The histological and ultrastructural observations on mouse kidney and liver which have been reported by FOWLER et al. [26] confirm both the cytotoxicity of carrageenan for Kupffer cells and the development of DIC following its administration. The 'blockade' of Kupffer cells by carrageenan [27] may also enhance DIC by inhibiting removal of fibrin, activated clotting factors and fibrin degradation products from the circulation [11]. Although the toxic effects described for carrageenan do restrict the usefulness of this agent, the extent of toxicity does vary with the preparation used. Indeed, iota carrageenan has been shown to inflict less severe damage on Kupffer cells than impure material [26].

Following its systemic injection carrageenan remains within liver and kidney for long periods. Six months after intravenous administration it can be demonstrated within Kupffer cells and in Bowman's spaces, collecting tubules and medullary pyramids of the kidney [26]. The persistence of carrageenan in the kidney results in chronic renal failure [26] with histological and ultrastructural features which suggest obstructive nephropathy.

7. Conclusions

Carrageenan impairs immune responses which are T-cell dependent and involve the participation of macrophages, towards which it appears to be selectively cytotoxic. This cytopathic effect on macrophages is a consequence of the ingestion of carrageenan by these cells and its persistence within secondary lysosomes. Due to its unique secondary and tertiary structure carrageenan is resistant to biochemical degradation by lysosomal glycosidases, and the persistence of high molecular weight material within phagolysosomes leads to osmotic swelling of these vacuoles and their eventual rupture. The consequent release of hydrolytic enzymes into the cytosol causes irreversible damage and eventual

cell lysis. Doubts concerning the specificity of the cytotoxic properties of carrageenan have emerged, but may be due to differences in the source, purity and concentration of the preparations employed. It is clearly important that the cytopathic properties of individual carrageenans are evaluated before their use as tools in studying the role of macrophages in immune reactivity.

Carrageenan also demonstrates anticomplementary activity directed against the early complement components, and although it has proved a potent immune suppressant, and can prolong graft survival in vivo, its use (at least in its present form) as an adjunct to existing immunotherapeutic manoeuvres cannot be contemplated. Systemic administration of carrageenan in laboratory animals results in disseminated intravascular coagulation and evidence of hepatotoxicity and nephrotoxicity. These properties clearly preclude its clinical use in organ transplantation.

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