Complement Activation by Alginate-Polylysine Microcapsules Used for Islet Transplantation

Sylviane Darquy, Maria E. Pueyo, Frederique Capron, and Gerard Reach

INSERM U, Service de Diabétologie, Hôtel-Dieu, Paris, France

Abstract: A foreign body reaction is frequently observed around implanted microcapsules of alginate-polylysine. Since complement activation can play a role in this reaction, we checked in vitro the ability of empty alginate-polylysine microcapsules to activate complement. Human serum was incubated with microcapsules, and complement activation was evaluated by two methods: the complement hemolytic activity ($CH_50$) and the assay of the C3adesArg fragment. The occurrence of complement activation in the presence of microcapsules was suggested both by a $CH_50$ decrease and by high C3adesArg levels despite C3adesArg adsorption to the capsule membrane. Capsule membrane protection against the cytotoxic effects of complement was also tested. No hemolysis occurred when microencapsulated sensitized sheep erythrocytes were incubated with activated complement.

In conclusion, the microcapsule membrane can protect cells against activated complement fragments. Nevertheless, alginate-polylysine microcapsules do activate complement, and this effect must be considered for its use as an implant.

Key Words: Microcapsules—Alginate-polylysine—Complement—Bioartificial pancreas.

Microencapsulation of islets of Langerhans inside an alginate-polylysine-alginate membrane (1) has been proposed for xenogeneic transplantation. Results of in vitro evaluation of these microcapsules were promising; the membrane was shown to protect islets against the cytotoxic effect of serum from insulin-dependent diabetic patients (2) and to permit adequate kinetics of glucose-stimulated insulin secretion by the encapsulated islets (3). However, in vivo studies of biocompatibility of the alginate-polylysine microcapsules have been performed, and foreign body reaction and fibrosis around the capsules have been reported by several investigators (4–6). Others, however, reported little or no fibrotic overgrowth around the implants (7). These contradictory results might be explained in part by the use of different recipient strains or species and different methods to quantify the tissue reaction around the microcapsules (8).

In order to avoid this bias, standardization procedures of evaluation would be required. Indeed, the fundamental objective in the analysis of material biocompatibility is an appreciation of the relationship between the implanted material and the host. The surface characteristics of the materials and the events at the tissue-biomaterial interface are critical for acceptance and durability of an implant (9). Certain biomaterials are known to interact with and activate the complement system (10), leading to the production of inflammatory mediators such as the C3a and C5a fragments which have a key role in the development of the foreign body reaction.

The aim of this work was to investigate in vitro the ability of empty alginate-polylysine capsules to activate the complement pathway of human serum. This in vitro approach to the development of the inflammatory reaction allows direct testing of the response of human complement to the microcapsules. In addition, we have evaluated the protective role of the microcapsule membrane against the activated terminal complex of complement.

STUDY DESIGN AND METHODS

Evaluation of human complement activation by empty alginate-polylysine capsules

Microencapsulation procedure

Empty alginate-polylysine microcapsules were prepared under sterile conditions, according to the...
COMPLEMENT ACTIVATION BY MICROCAPSULES

Effect of microcapsules on complement activation

The adsorption of complement proteins to the microcapsule membrane was evaluated.

**Preparation of human serum**

Blood from healthy donors was drawn into vacutainer tubes and allowed to clot at room temperature for 4 h. In order to remove natural hemolysins, 30 ml of serum was incubated with 1 ml of erythrocytes for 10 min at 0°C, then centrifuged 5 min at 2,000 rpm. This procedure was repeated twice. Serum was frozen at -80°C.

**Incubation of alginate-polylysine microcapsules with human serum**

To evaluate complement activation by alginate-polylysine capsules, 500 µl of human serum was incubated at 37°C in glass tubes in the presence of 50, 100, or 200 microcapsules. Serum without capsules was incubated as control to determine the spontaneous complement activity. Aliquots (50 µl) were removed at time 0 and at time 60 min for determination of the complement hemolytic activity and measurement of the C3adesArg fragment concentration. A decrease in complement activity during the experiment might reflect both complement activation by the capsules and complement adsorption to the capsule membrane. Similarly, an increase in C3a concentration due to complement activation might be masked by the adsorption to the capsule membrane. Thus, to assess complement activation, incubation was performed in parallel in two different buffers: veronal buffer saline (VBS), containing calcium and magnesium, in which complement activation does occur; and EDTA buffer, without calcium and magnesium, in which complement activation is inhibited. Variations of complement activity or concentration in EDTA buffer reflect only adsorption of complement proteins to microcapsule membrane. By comparing the values obtained with the two different buffers, complement activation by the capsules can therefore be evaluated.

**Determination of complement activation**

Two methods were used to assess complement activation. First, determination of the complement hemolytic activity was used. The complement hemolytic activity corresponds to the total functional activity of the classic pathway. The principle is to evaluate the capacity of normal human serum to provoke lysis of sheep antibody sensitized erythrocytes (erythrocyte-antibody, EA). The serum dilution leading to 50% of erythrocyte lysis (complement hemolytic 50 or CH50) is determined. Hemolysis intensity, which depends on the amount of the available complement in serum, can be readily quantified by the optical measurement of released hemoglobin. This method was used to assess the complement activity present in the serum before and 60 min after incubation with microcapsules. The preparation of sensitized erythrocytes for these assays is described elsewhere (12). Briefly, sheep erythrocytes (Diagnostic Pasteur, France) were incubated for 20 min at room temperature with a solution of rabbit antibodies anti-sheep erythrocytes (rabbit hemolytic serum; Diagnostic Pasteur). They were subsequently washed with VBS-EDTA 0.04 M buffer, and the concentration of cell suspension was brought up to 5.10⁶ cells/ml. Serial dilutions of sera (which had been previously incubated without or with microcapsules) were incubated with the sensitized erythrocytes in 5 ml glass tubes over 45 min in VBS medium. The incubation was interrupted by the addition of 0.15 M NaCl. Maximal hemolysis was determined by interrupting the reaction with distilled water. Optical density was determined in the supernatant after centrifugation for 10 min at 2,500 rpm. Results were first expressed for each serum as the percentage of maximal hemolysis. Finally, the serum concentration yielding 50% of maximal hemolysis (CH50) was determined.

Second, assay of C3adesArg was used also to assess complement activation. The concentration of the serum anaphylatoxin C3adesArg was measured before and after serum incubation with microcapsules. The assay was performed with a commercially available radioimmunoassay kit (Amersham, U.K.).

Evaluation of the protective role of the microcapsule membrane against the activated terminal complex of complement

The formation of transmembrane C5b-9 pores constitutes the primary mechanism of cytolysis by the complement. In order to verify the protection by the microcapsule membrane to terminal fragments generated during complement activation, microencapsulated sheep erythrocytes sensitized with anti-sheep antibodies were incubated at 37°C for 1 h with guinea pig serum and with Zymosan (1 mg/ml of serum; Sigma, St. Louis, MO, U.S.A.), a polysaccharide known to activate complement. Nonencapsulated sensitized erythrocytes were incubated under the same conditions as a positive control group. Hemolysis was determined by spectrophotometry at 595 nm. Microcapsulated sheep...
erythrocytes were prepared using the method proposed by Sun et al. (11).

**Expression of results and statistical analysis**

The complement activity in the serum after 1 h incubation was expressed as a percentage of the initial value observed at time 0 of the incubation. It represents the residual complement activity. C3adesArg concentration was also expressed as the increase over the initial value, expressed in percent. Hemolysis of sensitized sheep erythrocytes by guinea pig serum or Zymosan are expressed as a percentage of maximal hemolysis in presence of water.

Results in text and figures are expressed as mean ± standard error of the mean (SEM). Statistics are Student's *t* test and ANOVA. The level of significance was *p* < 0.05.

## RESULTS

**Human complement activation by empty alginate-polylysine capsules**

**Residual hemolytic complement activity**

A significant decrease (*p* < 0.05) in the complement activity (CH₅₀) was observed when serum was incubated in VBS with empty microcapsules, as compared with incubation without microcapsules (Fig. 1). This decrease in hemolytic activity could reflect a complement activation during incubation and/or a passive adsorption of complement proteins to the microcapsule membrane. However, in EDTA buffer, in which only adsorption could occur, the presence of microcapsules had no effect on the residual complement activity (Fig. 1). Furthermore, when the serum was incubated in the presence of microcapsules, the decrease in complement hemolytic activity observed in VBS medium was of greater magnitude than the decrease observed in EDTA buffer (*p* < 0.05). This suggests that the decrease in complement hemolytic activity was due to activation of complement and not to an adsorption to capsule membrane.

**C3a fragment production**

C3adesArg concentration was assessed in the serum before and after 60 min of incubation with microcapsules. Results at time 60 min were expressed as a percentage of the initial value (Fig. 2). C3adesArg concentration in serum incubated without microcapsules reflected the spontaneous C3a release, which as expected was higher in VBS medium than in EDTA buffer. The presence of capsules resulted in a significant decrease in C3adesArg concentration when the incubation was performed in EDTA buffer, a result consistent with an adsorption of C3a to the microcapsules. When the incubation was performed in VBS, a decrease in C3adesArg concentration was observed in preparations containing 50 or 100 microcapsules, but not in those containing 200 microcapsules. Taking into account the adsorption of C3adesArg revealed by the EDTA buffer experiments, these results suggest that in fact the presence of microcapsules stimulated C3a production.

**Evaluation of the protective role of the microcapsule membrane against the activated terminal complex of complement**

Sheep erythrocytes sensitized with anti-sheep antibodies were lysed during incubation with guinea pig serum, and the residual complement activity was expressed as a percentage of the initial value (time 0 of incubation).

![FIG. 1. Residual complement activity of serum incubated with and without microcapsules, in VBS medium (C2) or EDTA buffer (C6) is shown. Residual CH50 activity was expressed as a percentage of the initial value (time 0 of incubation): (a) Significantly different (p < 0.05) when comparing incubations with or without microcapsules. (b) Significantly different (p < 0.05) when comparing incubations in VBS medium and EDTA buffer containing the same number of microcapsules.](image)
pig serum (Fig. 3a). Such was not the case when microencapsulated sensitized erythrocytes were incubated irrespective of the guinea pig serum dilution. Similar results were obtained when Zymosan was added to the serum, demonstrating that even under maximal stimulation of complement activation, the erythrocytes were protected against the hemolytic activity of complement (Fig. 3b).

**DISCUSSION**

Following implantation of a biomaterial, the physicochemical and biological responses occurring at the interface host/implanted material and surrounding environment are of fundamental importance in determining the biocompatibility of the implant (13). The results of two different assays of complement activation, performed following incubation of microcapsules with human serum suggest that alginate-polylysine microcapsules can activate in vitro the complement system.

First, we observed a significant decrease in serum residual complement activity at the end of the incubation, when the experiments were carried out in VBS medium. By comparing these results with those obtained with EDTA buffer, which reflect only complement adsorption to the capsule membrane, the confounding bias of this adsorption could be excluded. Indeed, in the presence of microcapsules, the decrease in serum residual complement activity observed with VBS medium was of greater magnitude than the decrease observed with EDTA buffer ($p < 0.05$). Second, measurement of $\text{C3adesArg}$ concentration during these experiments confirmed the occurrence of both adsorption and complement activation in the presence of microcap-
sules. Adsorption of C3desArg was demonstrated by the decrease in C3desArg concentration during serum incubation in the presence of microcapsules in EDTA buffer. When the incubation was performed in VBS buffer, such a decrease was not observed, suggesting that it was actually masked by C3desArg production, overcoming the adsorption ability of the capsule membrane. This represents, therefore, indirect evidence for the activation of C3desArg production during serum incubation in the presence of microcapsules.

Activation of complement could be harmful for implanted microencapsulated islets of Langerhans. The most relevant products of complement activation with regard to biocompatibility are the anaphylatoxins C3a and C5a (14). In plasma, C3a and C5a are transformed into stable desArg derivates, which have chemotactic activity for neutrophils and monocytes and induce interleukin-1 (IL-1) release from monocytes (15). IL-1, with a molecular weight of 17,500 daltons (d), could cross the capsule membrane and have a deleterious effect on the islets. Indeed, this cytokine has been reported to produce functional and structural damage to isolated islet cells (16,17). IL-1 is also known to be involved in fibroblast proliferation (18) and could explain the fibrosis observed around implanted empty capsules by several authors (4,6,19).

However, the capsule membrane achieved a real protection against complement fragments. The terminal complement complex is generated by sequential assembly on target cell membranes of five precursors proteins (C5b, C6, C7, C8, C9) to form a multimolecular membrane attack complex. This complex inserts into cell membranes and exerts a lytic effect (20,21). Encapsulated cells were protected even in case of maximal complement activation. It must be stressed, however, that these incubations were not performed in the presence of macrophages. We have recently observed that alginate-polylysine microcapsules activate human macrophage to produce IL-1 (22). IL-1 (MW 17.5 kd) may cross the membrane, and kill the encapsulated islets since it is known to have a cytotoxic effect on islets of Langerhans (16,17).

The poor biocompatibility of some microcapsule preparations may be related either to alginate itself or to polylysine molecules not completely covered by alginate as discussed by De Vos (8). It has been reported that the alginate content in glucuronic and mannuronic acid may play a role in the biocompatibility of this biomaterial (23). Otherwise, purification of alginate from mitogenic contaminants may also improve its biocompatibility (24).  

The study of complement activation or another in vitro approach, like macrophage activation, presents some advantages, e.g., it allows testing of the reaction of human cells or human serum to the biomaterial. Furthermore, these studies are easily reproducible and quantifiable so that the comparison of responses to different materials might be easier. Whether alginate or polylysine or both materials are involved in the complement activation demonstrated in this study remains however speculative.

Encapsulated cells for transplantation are an interesting approach, but efforts must be made in the development of biocompatible membranes (25,26). Purification and changes in alginate composition or the use of other biomaterials that do not activate the complement system should be considered in the design of hybrid artificial organs.

Acknowledgments: We wish to express our gratitude to M. Kazatchkine, F. Maillet, and N. Haeffner-Cavaillon (INSERM U28) for fruitful discussions.

REFERENCES