

POLY (LACTIC/GLYCOLIC ACID) MICROSPHERES CONTAINING ANTIGEN
AS A NOVEL AND POTENTIAL AGENT OF IMMUNOTHERAPY
FOR ALLERGIC DISORDERSIppei Takagi, Jo Nishimura, Hirotaka Itoh and Shunkichi Baba
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In order to establish a safer and simpler antigen administration method in immunotherapy, we prepared biodegradable microspheres containing antigen and evaluated its safety and efficacy using guinea pigs. Poly (lactic/glycolic acid); (LGA) microspheres containing ovalbumin (OA) were fabricated by solvent evaporation. Over 70% of the OA was released from the microspheres within 3 days, and release was completed within 14 days in vivo. The local tissue reactions to the OA-LGA microspheres were apparently weaker than those to OA-alum. Repeated injections of high dose OA-LGA microspheres to OA-sensitized guinea pigs (high-LGA group) for 8 weeks at intervals of 2 weeks elicited an excellent therapeutic effect, i.e. a significant increase in the threshold value of antigen inhalation test, with a significant increase in IgG2 blocking antibody. The therapeutic efficacy of the high-LGA group was comparable to the conventional immunotherapy model (conventional group) and was superior to the antigen-alum model (alum group). We concluded that administration of antigen-LGA microspheres could become a new immunotherapeutic method for allergic disorders, being safer and requiring a lower frequency of antigen injections than the conventional method.

Key words: biodegradable microspheres — drug delivery system — immunotherapy — poly (lactic/glycolic acid) — repository antigen

INTRODUCTION

Although immunotherapy has been recognized as the most definitive therapeutic method for type I allergic disorders, allergic patients have been obliged to have frequent allergen injections, especially at the first therapeutic stage, in fear of various adverse reactions. For these reasons, conventional immunotherapy has not been widely carried out in most clinics. In order to make the immunotherapy more popular, a new antigen administering method which is safe and simul-

taneously requires fewer antigen injections should be introduced.

Recently many attempts to develop repository drug administering systems have been made, resulting in the establishment of a novel category of therapy, namely the drug delivery system (DDS). Poly (lactic/glycolic acid) (LGA) was first introduced as a bioabsorbable suture material in the 1960s. LGA had become a noteworthy drug reservoir material since it was clinically used for the administration of repository antinarcotic drug¹⁾. More recently LGA and its family have been applied as drug reservoirs in the DDS of hormone therapy²⁾⁻⁴⁾, antibiotic chemotherapy⁵⁾, and anticancer chemotherapy⁶⁾⁷⁾. LGA has the following chemical properties; it can be hydrolyzed gradually in vitro and in vivo without any enzymes, its hydrolysates, lactic acid and glycolic acid are non toxic to the organisms, and its hydrolytic rate can be varied according to its

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Abbreviations: DDS drug delivery system; ELISA enzyme-linked immunosorbent assay; LGA poly (lactic/glycolic acid); OA ovalbumin; PBS phosphate buffered saline; PCA passive cutaneous anaphylaxis

molecular weights, the ratio of lactic acid to glycolic acid, and other environmental factors⁸⁾⁹⁾.

The aim of this study was to develop a new antigen administration method which can extend the intervals of antigen injections with minimal local and systemic adverse reactions, using biodegradable microspheres consisting of antigen and LGA. The safety and efficacy of this method were evaluated in comparison with the conventional method and the alum-antigen method using immunotherapy models of guinea pigs.

MATERIALS AND METHODS

Antigen

Ovalbumin (OA, grade V, Sigma, MO, USA) as antigen was used for the preparation of OA-LGA microspheres, sensitization, immunotherapy, estimation of anti OA IgG1 and IgG2 antibodies, and 7-day PCA reaction. OA of grade III (Sigma) was used in the antigen inhalation test.

Biodegradable polymer

Poly (lactic/glycolic acid); (LGA) with a 1:1 ratio of lactic to glycolic acid and a molecular weight of 10000 daltons (LGA 5010) was purchased from Wako Pure Chemical Industrial Co. LTD., Osaka, Japan, and was used as an antigen reservoir throughout the experiments.

Antibodies

Anti OA antibody; rabbit anti chicken egg albumin serum was purchased from Seikagaku Kogyo Co. LTD., Tokyo, Japan. Anti OA antibody was purified with a protein A Sepharose column and biotinylated according to the method of Bayer and Wilchek¹⁰⁾.

Anti guinea pig IgG1 and IgG2 antibody; rabbit anti guinea pig IgG1 and IgG2 sera were purchased from Bethyl Laboratories Inc. TX, USA. The subclass specificity of these sera were confirmed by immunoelectrophoresis. The antibodies were purified, and biotinylated by the same method as described above.

Preparation of OA-LGA microspheres

Various amounts of OA in 16% gelatin solution were mixed with 50% LGA dichloromethane solu-

tion at a v/v ratio of 1:10 or 1:20. The mixture was then emulsified by blender (Ultra-Turrax, Janke and Kunkel KG, Germany), and subsequently by sonicator (ultrasonic disrupter UD200, Tomy Co. LTD., Japan) each for 1 min. Microspheres were produced by the solvent evaporation method¹¹⁾¹²⁾. The emulsified mixture was injected through a 22 or 23G needle into 0.2% poly vinyl alcohol solution with vigorous stirring. After further stirring to evaporate dichloromethane for 3 to 6 hr, fabricated microspheres were sieved through stainless steel meshes (No. 100, 280) to obtain microspheres of 50 to 150 μ m in diameter. The microspheres were then washed with deionized water, and were dried in vacuo. These procedures were performed at room temperature under sterile conditions.

Experimental animals

Guinea pigs of the Hartley strain (body weight: 250–300 g, 4 weeks old, both sexes) were used.

Sensitization of animals

OA-alum, which consisted of 10 μ g of OA and 5 mg of alum, prepared according to the method of Levine and Vaz¹³⁾ was injected intraperitoneally into the guinea pigs 4 to 6 times at intervals of 2 weeks. The success of sensitization was confirmed by antigen inhalation test.

Antigen inhalation test

The guinea pigs inhaled OA in physiological saline with serial twofold dilution by ultrasonic nebulizer in order of OA concentration (from lower to higher) for 2 min at intervals of 5 min. The minimal concentration of OA, which was recognized by a careful inspection to cause all of the following symptoms, namely, an apparent cyanosis, a relative prolongation of the expiratory phase, and a retraction of the chest wall at the inspiratory phase, was defined as the threshold value of the antigen inhalation test.

Evaluation of release of OA from OA-LGA microspheres

Two animals for each time course were used in this experiment. One hundred mg of OA-LGA microspheres containing 25 μ g of OA were suspended in 1 ml of physiological saline containing 0.1% Tween 80, and 1% carboxymethyl-cellulose.

This solution was used to make the suspension of LGA microspheres for injections throughout the study. The suspensions were then injected subcutaneously into OA-sensitized guinea pigs. The microspheres were surgically collected from the injection site after 1, 3, 7, 14 and 21 days. The OA remaining in the collected microspheres was extracted by dissolving the polymer matrix in 100% dimethyl sulfoxide. The OA amount in the extract was estimated by ELISA¹⁴⁾. A microplate with 96 wells (Nunc, Denmark) was coated with 50 μ l/well of rabbit anti OA serum diluted 200-fold with phosphate buffered saline (PBS). Following the blocking of nonspecific binding by the addition of 1% casein, 50 μ l/well of the sample solutions or OA solutions with variable concentration were added duplicately and incubated. After the microplate was washed with PBS containing 0.1% Tween 20, 50 μ l/well of biotinylated anti OA antibody solution was added and incubated. Following further washing, 50 μ l/well of avidin- β -galactosidase (ZYMED Laboratories, Inc., CA, USA) was added. As a substrate, 100 μ l/well of 0.3 mM methylumburyferyl- β -D-galactopyranoside (KOCH-LIGHT LTD., UK) was used. The enzyme-substrate reaction was terminated by the addition of 100 μ l/well of 0.5 M glycine-NaOH buffer (pH 10.5). The fluorescence intensity of the sample well was estimated with an MTP 96 well microplate reader (Corona Co. Ltd., Japan). Finally, OA concentration was calculated according to the standard curve made by OA solutions with known variable concentrations. Each antigen-antibody reaction was performed for 2 hr, and each avidin-biotin reaction for 15 min at room temperature throughout the experiments.

Seven-day PCA (passive cutaneous anaphylaxis) reaction¹⁵⁾

Sera from OA-sensitized guinea pigs, with serial twofold dilution with physiological saline, were injected intradermally to non-sensitized guinea pigs. After 7 days, 1 ml of physiological saline containing 1 mg of OA and 10 mg of Evans blue was administered intravascularly. After thirty minutes, the animals were sacrificed. The maximum dilution ratio of the serum which made the dye permeate the skin with a diameter of at least 7 mm was defined as the titer. Each serum was

titrated using two animals, and the logarithm of the higher titer in the two was defined as the final titer of the 7-day PCA reaction.

Estimation of anti OA IgG1 and IgG2 antibody

The titers of specific IgG1 and IgG2 antibodies against OA in the sera obtained from the guinea pigs were measured by ELISA. In brief, the microplate was coated with 50 μ l/well of OA (1 μ g/ml), nonspecific bindings were blocked with casein, and 50 μ l/well of the diluted serum was then added and incubated. After the microplate was washed, 50 μ l/well of biotinylated anti guinea pig IgG1 or IgG2 antibody was added and incubated. Following further washing, avidin- β -galactosidase was added. Enzyme-substrate reaction was done in the same manner as the above-mentioned measurement for OA concentration. The specific antibody titer was arbitrarily defined as the logarithm of the ratio of fluorescence intensity of the sample to the intensity of one thousandfold diluted reference sera obtained from OA sensitized guinea pigs.

Experimental immunotherapy using guinea pigs (Fig. 1)

OA-sensitized guinea pigs were divided into 5 groups, namely control-LGA group, low-LGA group, high-LGA group, alum group, and conventional group. The low- and high-LGA groups were subcutaneously injected with OA-LGA microspheres (1.7 μ g OA/mg microspheres) for 8 weeks at intervals of 2 weeks (Fig. 1(a)). The control group was given the same weight of microspheres as the high-LGA group but without OA. The alum group received subcutaneous injections of an increasing dose of OA-alum (OA 30–150 μ g, alum 5–10 mg) for 8 weeks at intervals of 2 weeks (Fig. 1(b)). The conventional group received subcutaneous injections of OA in physiological saline twice a week for the initial 5 weeks, then once a week for the latter 5 weeks (Fig. 1(c)). The total amounts of OA administered were 217 μ g in the low-LGA group, 567 μ g in the high-LGA group, 570 μ g in the alum group, and 580 μ g in the conventional group.

Statistics

Paired *t* test was used for the statistical analysis.

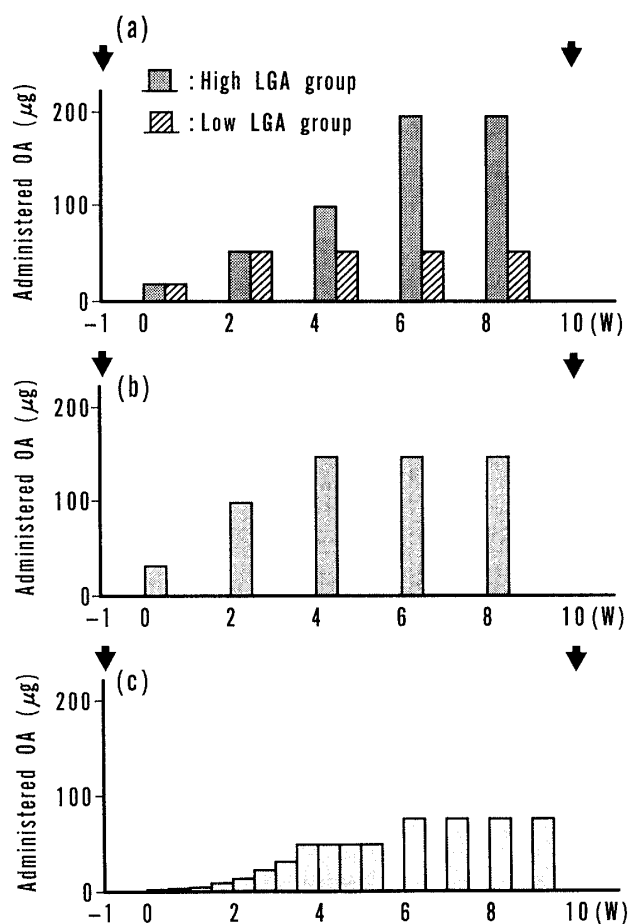


Fig. 1. Schedule of immunotherapy.

(a): High-LGA and low-LGA group. Control-LGA group was given LGA microspheres alone, with the same weight as the high-LGA group. (b): Alum group. (c): Conventional group. Total amounts of OA administered as antigen were 217 μg in the low-LGA group, 567 μg in the high-LGA group, 570 μg in the alum group, and 580 μg in the conventional group. Arrows indicate blood sampling and antigen inhalation test performed before and after immunotherapy.

sis of the paired data from the same group. Two sample *t* test was used to compare the data from two independent groups.

RESULTS

Release of OA from OA-LGA microspheres

OA was released from OA-LGA microspheres in a two-phased pattern (Table 1). The mean amount of OA in the microspheres injected into the dorsal subcutaneous tissue of the guinea pigs decreased

Table 1 %OA remaining in the collected microspheres

Animal No.	Days after injection				
	1	3	7	14	21
1	42.9				
2	28.6				
3		27.1			
4		25.7			
5			27.1		
6			27.9		
7				<1.0	
8				<1.0	
9					<1.0
10					<1.0
Mean	35.8	26.4	27.5	<1.0	<1.0

Microspheres were surgically collected from the dorsal subcutaneous tissue of guinea pigs 1, 3, 7, 14 and 21 days after injection. The amount of OA remaining in the microspheres was estimated by ELISA. Animals were used in duplicate for each time course. Numbers show the percentage of the amount of OA remaining in the collected microspheres.

rapidly to 26.4% within 3 days, notably to 35.8% in one day. No obvious decrease in OA was then observed until the 7th day. OA finally disappeared from the collected microspheres within 14 days.

Tissue reaction of injection site to OA-LGA microspheres

Two animals for each time course were used in this experiment. The injection sites were observed macro- and microscopically after subcutaneous injections of OA-LGA microspheres (100 mg of microspheres containing 25 μg of OA) into OA-sensitized guinea pigs. Only slight edema and bleeding of the surrounding tissue was observed macroscopically within 3 days after the injection. After 7 days, no macroscopic inflammatory reaction in the surrounding tissue was found. There was no difference in the tissue reactions between the injection sites of OA-LGA and control LGA microspheres (the same weight of LGA microspheres without OA). The microspheres completely disappeared from the injection sites by 3 to 4 weeks without any prolonged tissue reactions. In the microscopic observation, slight infiltration of neutrophils, macrophages, and lymphocytes into the tissue surrounding the microspheres was

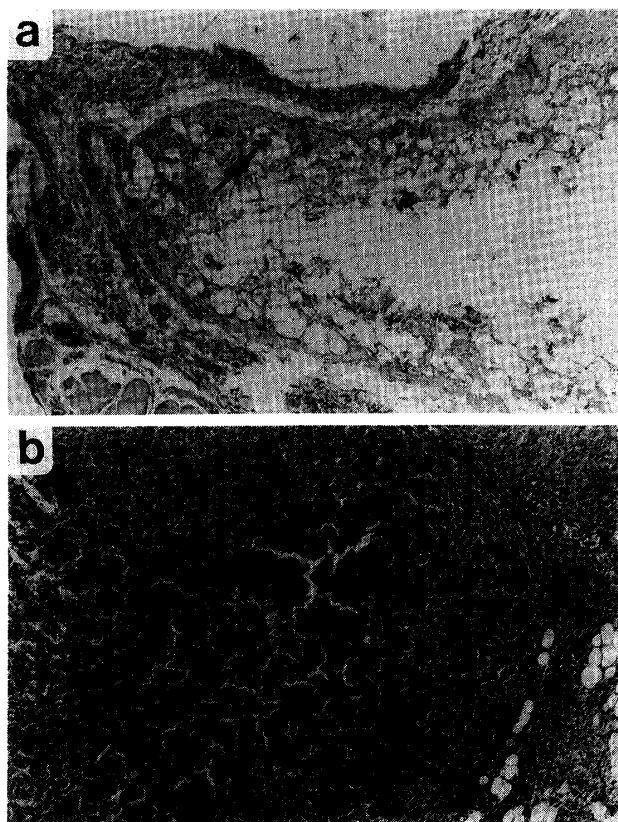


Fig. 2. Microscopic findings of subcutaneous tissue after the injection of OA-LGA microspheres or OA-alum (HE staining, $\times 75$).

(a): Typical findings of the tissue 7 days after the injection of OA-LGA microspheres (OA 25 μg in 100 mg of microspheres) into OA-sensitized guinea pig. Most of the microspheres were dissolved during the preparation of the paraffin sections. Arrow indicates the microspheres remained. (b): Typical findings of the tissue 7 days after the injection of OA-alum (OA 25 μg , alum 100 mg). Arrow indicates the alum crystal.

recognized within 7 days. Fig. 2(a) shows typical microscopic findings of the subcutaneous tissue 7 days after the injection of OA-LGA microspheres. After 14 days, a moderate accumulation of macrophages which phagocytized the debris of the microspheres was dominant. On the other hand, the tissue reaction to OA-alum was extremely severe in comparison with the reaction to OA-LGA microspheres. When the same dose of OA (25 μg) with 100 mg of alum was injected subcutaneously into OA-sensitized guinea pigs, granulation and abscess formation persisted macroscopically in the injection site for at least 3 months. Fig. 2(b) shows typical microscopic findings of the sub-

cutaneous tissue 7 days after the injection of OA-alum. A large number of neutrophils and macrophages were infiltrated into the tissue surrounding the crystals of alum. The severe inflammatory reactions persisted microscopically for at least 3 weeks. When a higher dose of OA-LGA microspheres (100 mg of microspheres containing 250 μg of OA) was injected into OA-sensitized guinea pigs, a small number of eosinophils were infiltrated around the microspheres within 3 days, which corresponded well to the period of rapid OA release from the microspheres.

Systemic reaction to OA-LGA microspheres

There was no remarkable systemic reaction, such as anaphylactic shock or respiratory distress, due to the injection of OA-LGA microspheres in any animals throughout the experiments in this study, even when a considerably high dose of OA-LGA microspheres (100 mg of microspheres containing 500 μg of OA) was injected daily for 6 days into three OA-sensitized guinea pigs.

Therapeutic efficacy of OA-LGA microspheres on immunotherapy models

(1) Antigen inhalation test (Table 2); The mean threshold values of the control-, and low-LGA group decreased after immunotherapy, but without statistical significance. Contrarily the high-LGA group showed a statistically significant increase in the mean value after immunotherapy ($p < 0.01$). The alum group showed an increased mean value, but without statistical significance. The conventional group showed a statistically significant increase in the mean value after immunotherapy ($p < 0.05$). There was no significant

Table 2 Changes in the threshold values of antigen inhalation test

Group	n	Before	After	Statistics
Control	5	2.0 ± 1.12	0.32 ± 0.12	N.S.
Low-LGA	5	1.44 ± 0.47	0.68 ± 0.25	N.S. ($p = 0.056$)
High-LGA	6	1.0 ± 0.20	10.93 ± 1.87	$p = 0.0024$
Alum	6	1.13 ± 0.46	7.07 ± 2.60	N.S. ($p = 0.084$)
Conventional	6	1.63 ± 0.98	8.07 ± 2.25	$p = 0.026$

Numbers show mean threshold values (mg OA/ml) $\pm$ S.E.
N.S.: not significant.

Table 3 Changes in the titers of 7-day PCA reaction

Group	n	Before	After	Statistics
Control	5	1.02±0.44	2.20±0.21	N.S.(p=0.0925)
Low-LGA	5	1.02±0.47	2.23±0.28	p=0.046
High-LGA	6	1.22±0.41	1.80±0.22	N.S.
Alum	6	1.44±0.30	1.55±0.052	N.S.
Conventional	6	1.27±0.41	1.85±0.12	N.S.

Numbers show mean final titers (logarithms of the titers)±S.E. N.S.: not significant.

Table 4 Changes in the titers of anti OA IgG1 antibody

Group	n	Before	After	Statistics
Control	5	3.28±0.26	2.97±0.18	N.S.(p=0.072)
Low-LGA	5	2.07±0.29	3.44±0.30	p=0.0001
High-LGA	6	2.34±0.47	3.13±0.56	N.S.
Alum	6	2.22±0.30	3.09±0.44	N.S.(p=0.070)
Conventional	6	3.19±0.43	2.41±0.35	N.S.(p=0.077)

OA specific IgG1 antibody was estimated by ELISA. The titer was arbitrarily defined as logarithm of the ratio of fluorescence intensity of the sample to the intensity of one thousandfold diluted reference sera obtained from OA sensitized guinea pigs. Numbers show mean titers±S.E. N.S.: not significant.

difference between the mean values of high-LGA and conventional group after immunotherapy.

(2) Seven-day PCA reaction (Table 3); The control-LGA group revealed an increased mean titer in the 7-day PCA reaction after immunotherapy, but without statistical significance. The low-LGA group showed a statistically significant increase in the mean titer after immunotherapy ($p<0.05$). The other three groups showed no significant changes in the mean titers after immunotherapy.

(3) Anti OA IgG1 antibody (Table 4); The control-LGA and conventional group showed decreased mean titers of anti OA IgG1 antibody after immunotherapy, but without statistical significance. The low-LGA group showed a statistically significant increase in the mean titer after immunotherapy ($p<0.001$). The alum group and high-LGA group showed increased mean titers, but without statistical significance.

(4) Anti OA IgG2 antibody (Table 5); The control-LGA group revealed a significantly de-

Table 5 Changes in the titers of anti OA IgG2 antibody

Group	n	Before	After	Statistics
Control	5	2.42±0.12	1.63±0.086	p=0.0036
Low-LGA	5	1.47±0.36	2.68±0.31	p=0.013
High-LGA	6	1.82±0.34	2.79±0.22	p=0.042
Alum	6	1.46±0.32	2.12±0.14	N.S.(p=0.052)
Conventional	6	1.82±0.24	2.91±0.068	p=0.010

OA specific IgG2 antibody was estimated by ELISA. The titer was arbitrarily defined by the same way as the titer of anti OA IgG1 antibody. Numbers show mean titers±S.E. N.S.: not significant.

creased mean titer of anti OA IgG2 antibody after immunotherapy ($p<0.01$), while the low-LGA, high-LGA and conventional groups showed significant increases in the mean titers after immunotherapy ($p<0.02$, $p<0.05$, $p<0.02$ respectively). The alum group showed an increased mean titer after immunotherapy, but without statistical significance.

DISCUSSION

Various techniques to overcome the disadvantages of conventional immunotherapy have been introduced these several decades. They can be divided into two large groups. In one, the allergenicity is reduced while retaining the immunogenicity of allergen molecules by chemical modifications, such as denaturation^{16)–18)}, polymerization of the allergens¹⁹⁾²⁰⁾ and conjugation of the allergens with non-immunogenic polymers^{21)–23)}. In the other, the allergens are invested with repository properties based on the theory of DDS. The latter group consists of oil-emulsified allergens²⁴⁾, alum-precipitated allergens^{25)–27)}, allergens adsorbed to insoluble amino acid^{27)–29)}, and most recently liposome-entrapped antigens^{30)–32)}. However, up to the present, no ideal method has been developed to completely obviate the disadvantages of the conventional method. In this study, as one of the latter approaches, a new technique using antigen-containing biodegradable microspheres was introduced.

The fabricated antigen (OA)-LGA microspheres released OA for 7 to 14 days in vivo. A considera-

bly large amount of OA was released from the microspheres in the first 3 days, especially within 24 hours of the injection. The result is attributable to the bursting of part of the microspheres by the low osmotic pressure in the environment prior to the actual hydrolysis of the polymer matrix. Although this bursting of the microspheres might result in the occurrence of a serious anaphylactic reaction, virtually none of the animals which were given OA-LGA microspheres showed any systemic reaction throughout the experiments. The results indicates that the release of OA from the microspheres in the early period is not so explosive as to induce any anaphylactic reaction. We confirmed that the amount of OA released from the microspheres in the first half of the first 24 hours was comparable to that in the latter half (unpublished data). Gelatin, which is used to prevent the denaturation of antigen molecules during the emulsification with organic solvent, can probably invest the antigen with a repository property even after the bursting of the microspheres. Furthermore, the microspheres have a diameter of at least 50 μm . This size is large enough to prevent the microspheres from entering the microcirculation.

The macro- and microscopic findings at the local injection sites also supported the safety of antigen-LGA microspheres. OA-LGA microspheres elicited only mild local inflammatory reactions, while OA-alum induced severe, persistent inflammation including abscess formation in the surrounding tissue. Type I allergic reaction in the tissue surrounding the OA-LGA microspheres, which was evidenced by the accumulation of eosinophilic leucocytes, was observed only during the early period in which a relatively large amount of antigen was released. The administration of alum-precipitated antigen, which had already been used in immunotherapy as a repository antigen²⁶⁾²⁷⁾, was reported to produce persistent subcutaneous induration in some child cases³³⁾. Moreover, the inflammatory reaction is presumed to be caused by delayed type hypersensitivity to aluminum³³⁾. Our results also indicate that alum is extremely harmful as an antigen carrier to the injection site. The antigen-LGA microspheres macroscopically disappeared from the injection site without any granulation or scar formation. The safety of antigen-LGA microspheres in the

injection site especially suggests a possible facilitation of repeated antigen injections without cumbersome local adverse reactions in the clinical situation.

The therapeutic efficacy of the administration of antigen-LGA microspheres was evaluated in immunotherapy models using guinea pigs. Repeated administrations of OA-LGA microspheres with a high dose (high-LGA group) to OA-sensitized guinea pigs showed an excellent therapeutic effect, i.e. a significant increase in the mean threshold value in the antigen inhalation test after 8 weeks. Unsatisfactory results of low-dose administration of OA-LGA microspheres (low-LGA group) was considered to be due to an undesirable increase in reaginic IgG1 antibody and IgE antibody (evidenced by an increased mean titer in the 7-day PCA reaction) despite the increase in IgG2 antibody. This can be explained by the theory in immunology that long term administration of low doses, in comparison with high doses of antigen, easily induces reaginic antibodies rather than other specific antibodies¹³⁾. Compared with other antigen administering methods, the administration of antigen-LGA microspheres (high-LGA group) showed a comparable therapeutic efficacy to that of the conventional immunotherapy model (conventional group) and showed a superior efficacy to that of antigen-alum (alum group) when the total amounts of administered antigen were made approximately equal. These results imply certain advantages of antigen-LGA microspheres, namely the excellent therapeutic efficacy and the requirement of fewer antigen injections, as a promising agent of immunotherapy for allergic patients. Furthermore, the high-LGA group and the conventional group succeeded in inducing significant amounts of antigen specific IgG2 antibody without any significant increase in reaginic IgG1 and IgE antibody. Since IgG2 antibody is non-homocytotropic and is also known as a blocking antibody in atopic guinea pigs³⁴⁾, the increase in IgG2 antibody, without significant change in reaginic antibodies, must result in the increase of the threshold value in the antigen inhalation test. The lack of any significant increase in IgG2 antibody in the alum group might indicate the inferiority of alum as an antigen carrier in immunotherapy because of its

property of inducing dominant reagin production in the immunization of animals¹³⁾¹⁵⁾. As reported by Holzman et al.³⁵⁾, alum-precipitated antigen produces prolonged retention of the antigen at the injection site if a sufficiently high alum to antigen ratio was used. This property of alum-antigen is not favorable in the acceleration of the secondary antibody response, including the production of the blocking antibody.

On the other hand, significant accumulation of macrophages ingesting the debris of OA-LGA microspheres suggests that presence of active antigen recognition by the mononuclear phagocyte system, resulting in a good induction of specific IgG2 antibody. Tabata et al.³⁶⁾ reported that LGA and its related polymers could be phagocytized well by macrophages. In addition, gelatin contained in the microspheres with antigen can also enhance the production of blocking antibody since it has been known as an easily ingestible material for phagocytes³⁶⁾³⁷⁾.

Most recently, with a similar motive to our study, Arora and Gangal³⁰⁾³¹⁾, Audera et al.³²⁾ reported independently that the administration of liposome-entrapped antigen to mice produced an inhibition of the primary IgE response despite an enhanced IgG response in comparison with the administration of antigen alone. Liposome-entrapped antigen as well as antigen-LGA microspheres may prevent the adverse reactions accompanying immunotherapy because of its repository characteristics. However, according to the variable requirement of each case, antigen release is more controllable in our method than in liposome-entrapped antigen because of the variability of the hydrolytic rates of LGAs⁸⁾⁹⁾. In addition, in our study, the therapeutic efficacy of antigen-LGA microspheres administration could be shown through secondary immunoresponses, including the blocking antibody production, in animals with an ongoing, atopic state.

In conclusion, antigen-LGA microspheres may become a new agent for an improved immunotherapy for allergic disorders through the following unique characteristics: weaker local adverse reactions than antigen-alum, excellent therapeutic efficacy with satisfactory induction of the blocking antibody, and finally the requirement of fewer antigen injections than the conventional

method.

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抗原を含有するポリ（乳酸/グリコール酸）マイクロスフィア —新しい減感作療法剤としての基礎的検討—

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従来の減感作療法に比べて安全で、少ない抗原投与頻度で効果が得られる抗原投与方法開発のため、抗原含有の生体内分解性マイクロスフィアを作製し、モルモット減感作療法モデルにおいて基礎的検討を行った。抗原（ovalbumin, OA）と生体内分解性ポリマー（poly（lactic/glycolic acid）, LGA）よりなるマイクロスフィア（OA-LGA MS）を液中乾燥法にて作製し、OA 感作モルモット皮下に投与し、局所全身の安全性、治療効果を評価した。in vivo で OA-LGA MS は 7～14 日間に亘って OA を徐放し、特に投与 3 日以内に大部分が放出された。OA-LGA MS に対する局所組織反応は OA-alum に比べて極めて軽微であった。OA 感作モルモットに対して OA-LGA MS を 2 週毎、8 週間漸増投与（high-LGA 群）した結果、OA 吸入試験での閾値および OA 特異的 IgG2 抗体価の有意の上昇を認めた。これらの結果は OA-alum 投与（alum 群）より優れ、OA 単独頻回投与（conventional 群）とほぼ同等であった。以上より抗原-LGA MS は安全で抗原投与間隔を延長できる新しい減感作療法剤として期待できると結論された。
