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Generation of IgG Polymers by the Myeloperoxidase-H_2O_2-Catechol System and Their Stimulatory Effect on Oxygen Radical Production from Human Polymorphonuclear Leukocytes[1]

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= Abstract =Polymerization of native human IgG by myeloperoxidase (MPO)-H_2O_2-catechol sytem and ability of the resulting polymers to stimulate polymorphonuclear neutrophils (PMNs) to produce oxygen radicals were studied.

Incubation of the human IgG with PMN granules (as a source of MPO) and H_2O_2 in the presence of catechol as a hydrogen donor resulted in polymerization of this protein, which was confirmed by Sephacryl 200 column chromatography and polyethylene glycol precipitation method. The polymer formation was completely abolished by omission of PMN granules, H_2O_2 or catechol, and addition of catalase or azide to the complete system containing the three components. Among the various hydrogen donors tested, only dopamine was able to replace catechol in the polymerization (about 75% of that of catechol). The polymerization was not affected by the quenchers for O_2^-, OH· or $^1O_2$ and antioxidants but markedly inhibited by benzenesulfinic acid and some compounds acting as a hydrogen donor. These results suggest that the polymerization was due to oxidation of catechol to orthoquinone mediated by MPO and H_2O_2 with ruling out the involvement of the oxygen radicals.

The IgG polymers obtained stimulated human PMNs to proudce O_2^- and H_2O_2 while native IgG was much less effective in this respect. The stimulatory effect was even higher than (about 1.5 times) that of heat-aggregated IgG having many properties similar to typical immune complexes. Thus, it can be expected that the IgG polymers generated in the present study will also behave as immune complexes in stimulation of PMNs.

The result was discussed in terms of a possible role of the IgG polymers in the pathogenesis of tissue injury in many acute and chronic inflammatory conditions.

Key words: ***Immunoglobulin G(IgG) polymers, Myeloperoxidase(MPO)-H_2O_2- catechol system, Oxygen radicals***

INTRODUCTION

Immune complexes (IC_s) are involved in the pathogenesis of inflammatory diseases of immunologic origin (Cochrane and Koffler 1973). Various mechanisms have been suggested for the actions of IC_s under these conditions. For exmples, IC_s can amplify inflammatory responses via the generation of chemotactic peptides through the activation of complement system (Theofilopoulous and Dixon 1979; Petrone *et al.* 1980). They can also stimulate polymorphonuclear leukocytes (PMN_s) to release a number of lysosomal proteases hydrolyzing major components of connective tissues, such as collagen and elastin, ultimately leading to tissue

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damage (Weissmann *et al.* 1979). In addition to the enzyme release, IC_s activate PMNs to produce reactive oxygen species such as superoxide anion ($O_2^{\cdot-}$) and hydrogen peroxide (H_2O_2) extracellularly (Fantone and Ward 1982; Weiss and LoBuglio 1982; Weiss and Ward 1982). Because of their reactivity, they also attack and destroy the tissue components (McCord 1974; Greenwald and Moy 1979; Chung *et al.* 1984). In view of the many reports suggesting roles of the reactive oxygen species in the tissue injury in inflammatory conditions (Fantone and Ward 1982), the generation of the oxygen species by IC_s can be also an important mechanism in the ICs-mediated tissue injury.

The reactive oxygen species can exhibit toxicites in many ways. But as far as PMN-involved oxygen-dependent tissue damage is concerned, peroxidation by myeloperoxidase (MPO), a lysosomal enzyme of PMN_s, may be a major mechanism using H_2O_2 as a substrate and halides as cofactors. Although the MPO-mediated peroxidation primarily serves as a microbicidal mechanism against phogocytized bacteria (Klebanoff 1980), the activated PMNs can exert extracellular toxicities using reactive oxygen species, but most of them occur through the MPO-mediated peroxidation, for example, lysis of mammalian cells (Clark and Klebanoff 1977), killing of hyphal forms of fungal pathogens (Diamond *et al.* 1980), and inactivation of enzymes (Jin *et al.* 1986) and serum factors such as α_1-protease inhibitor (Clark *et al.* 1981).

In addition to the toxic effect mentioned above, it was also shown that MPO can polymerize immunoglobulin G(IgG) in the presence of hydrogen donors such as catechol (Jasin 1983). On the other hand,the production of the oxygen species from PMN_s can be triggered by various stimuli, i.e., phagocytic, chemical and immunologic. In the immunologic response, IC_s such antigen-antibody complexes may act as natural stimuli but PMN_s also respond to non-specifically polymerized IgG such as heat-aggregated IgG (Ishizaka and Ishizaka 1959; Johnson and Lehmeyer 1976).

Based upon this observation, it is expected that the IgG polymers formed by the MPO-mediated peroxidation can stimulate PMN_s to produce reactive oxygen species. If this is true, the IgG polymers may also play a role in the PMN-induced oxygen-dependent toxicities as IC_s can do through the activation of PMN_s.

In the present study, human IgG was polymerized by MPO/H_2O_2/catechol system and the resulting polymers were evaluated for the ability to stimulate PMN_s to produce the oxygen species. In this study it was observed that the IgG polymers stimulated PMN_s to produce reactive oxygen species, $O_2^{\cdot-}$ and H_2O_2. The result obtained suggests possible involvement of the polymers in oxygen-dependent tissue injury and this may be an additional role of MPO in the toxicities induced by PMNs.

MATERIALS AND METHODS

Dextran (M.W., 250,000), ethylenediamine tetraacetic acid (EDTA), human IgG, catechol, dopamine, epinephrine, dopa, ascorbic acid, glutathione, uric acid, α-tocopherol acetate, polyethylene glycol (M.W., 6,000), superoxide dismutase (SOD), catalase, cytochrome C, horse radish peroxidase (HRP) and homovanillic acid (HVA) were obtained from Sigma Chem. Co.; Sephacryl 200 and Percoll from Pharmacia Fine Co.; sodium azide, dimethyl sulfoxide (DMSO) from Merck; benzenesulfinic acid from Aldrich Chem. Co.; H_2O_2 from Shinyo Pure Chem. Co.; mannitol from Hayashi Pure Chem. Co., and other chemicals are of analytical reagent grade.

1. Preparation of human polymorphonuclear leukocytes

Human PMN_s were prepared from venous blood of healthy adult volunteers by the Percoll discontinuous density gradient method described by Giudicelli *et al.* (1980). Briefly, 20 ml of heparinized blood (10 I.U./ml) were mixed with 2 ml of 10% EDTA, pH 7.4 and diluted with an equal volume of phosphate-buffered saline (PBS), pH 7.4. The mixture was carefully layered on top of the discontinuous Percoll gradient prepared with 10 ml of 80% Percoll and 10 ml of 60% Percoll and centrifuged at 600g for 30 min. Of two distinct leukocyte layers obtained, the lower layer was harvested and suspended in cold distilled water for 20 sec to lyze contaminating erythrocytes and then 1.5N NaCl was added to bring the suspension to isotonic strength. The cells were recentrifuged and the above lysis procedure was followed until no erythrocyte remained. All the procedures of PMN preparation were performed at room temperature. The PMN_s obtained were suspended at a concentration of 1 to 2 $\times$ 10^7 cells/ml in PBS, pH 7.4 supplemented with 1g/l glucose and kept in ice until used. The preparation contained 95% or more granulocytes and more than 90% viable cells as evaluated by trypan blue staining.

2. **Preparation of the PMN granules as a source of myeloperoxidase**

Blood was mixed with an equal volume of 3% dextran and allowed to sediment for 45 min at room temperature (Böyum 1968). Buffy coat was collected and used to prepare the PMNs by the Percoll discontinuous gradient method as described above. PMN granules were isolated from the PMN_s obtained by the method of Chodirker *et al.* (1968) and stored at −20°C until used. Peroxidase activity of the PMN granules was determined by the method of Maehly and Chance (1954). One unit was defined as the activity decomposing 1 μ mole H_2O_2/min at 25°C.

3. **Polymerization of human IgG by the MPO-H_2O_2-catechol system**

Native human IgG (4 mg protein/ml) was incubated with the PMN granules (40 munit/ml) as a source of MPO in 0.5 ml PBS, pH 7.4 containing various concentrations of H_2O_2 and catechol. The reaction was performed in the dark for 60 min at 37°C with continuous agitation and stopped by adding sodium azide to give a final concentration of 2 mM. The reaction mixture was used for identification and quantitation of IgG polymers formed.

4. **Analysis of IgG polymers generated by the MPO-H_2O_2-catechol system**

IgG polymers produced by the MPO-H_2O_2-catechol system were identified by gel permeation chromatography and quantitated by precipitation method with polyethylene glycol (PEG; M.W., 6,000) (Creighton *et al.* 1973; D'amelio *et al.* 1979). The reaction mixture described in the previous section was spun down at 150g for 5 min. An aliquot (0.2 ml) taken for the supernatant was loaded on a Sephacryl 200 column equilibrated with PBS, pH 7.4 containing 1% sodium azide. The column was eluted at room temperature with the same buffer at a flow rate of 8 ml/h and eluent was continuously monitored at 280 nm. For the quantitation of IgG polymers, another aliquot (0.2 ml) from the supernatant was mixed with an equal volume of 4% PEG in PBS, pH 7.4 and incubated for 21h at 4°C. The mixture was centrifuged at 1,500g for 30 min at 4°C and the supernatant was removed. The precipitate was dissolved in 0.5 ml of 0.1N NaOH and the protein content was determined by the method of Lowry *et al.* (1951) with human IgG used as the standard.

5. **Assay of superoxide (O_2^-) and hydrogen peroxide (H_2O_2) produced from human PMN_s stimulated by various IgG**

Native IgG, IgG polymers formed by the MPO-H_2O_2-catechol system or heat-aggregated IgG were compared for the activity of stimulating PMN_s to produce O_2^- and H_2O_2. Both IgG polymers formed by the MPO system and heat-aggregated IgG prepared by heating native IgG at 63°C for 30 min were concentrated with Centriflo CF 5A membrane cone (Amicon). The concentrated IgGs were used in this experiment.

The production of O_2^- was assayed by the reduction of cytochrome C (Nakagawara *et al.* 1976). Human PMN_s (10^6 cells/ml) were suspended in PBS, pH 7.4 containing 1 gm/l glucose, 0.9 mM Ca^{++}, 0.5 mM Mg^{++} and 50 μM cytochrome C. The reaction mixture was transfered to a thermostated cuvette at 37°C which was placed in an Aminco-Chance dual wavelength spectrophotometer and incubated for 5 min. The reaction was started by adding one of the above IgGs. The volume of the reaction mixture was adjusted to 1 ml when the each IgGs was added last. The absorbance change at 550-540 nm was used to calculate the amount of cytochrome C reduced as an index of O_2^- production with a molar absorption coefficient (reduced·cytochrome c minus oxidized one) of $19.1 \times 10^3\ M^{-1}\ cm^{-1}$.

The production of H_2O_2 was determined by the increase of fluorescence due to the oxidation of homovanillic acid (HVA) catalyzed by horse radish peroxidase (Takeshige *et al.* 1979). Human PMNs (10^6 cells/ml) were suspended in PBS, pH 7.4 containing 1 g/l glucose, 0.9 mM Ca^{++}, 0.5 mM Mg^{++}, 5 μg/ml HRP and 0.5 mM HVA. The suspension was incubated for 5 min in a thermostated cuvette at 37°C which was placed in a Perkin-Elmer fluorometer and the reaction was started by adding one of the above IgGs. The amount of H_2O_2 produced was calculated by comparing the fluorescence change observed with that caused by known amounts of H_2O_2.

RESULTS

1. **Polymerization of IgG by the MPO-H_2O_2-catechol system**

Native IgG showed a single peak on the gel permeation chromatography (Fig. 1.A). When the IgG was treated with PMN granules (40 munit/ml), 1 mM H_2O_2 and 1.25 mM catechol, an additional new peak was observed, which was eluted ahead of the normal IgG peak (Fig. 1.B). But when the treatment of the IgG with PMN granules, H_2O_2 and catechol was done in the presence of 2 mM

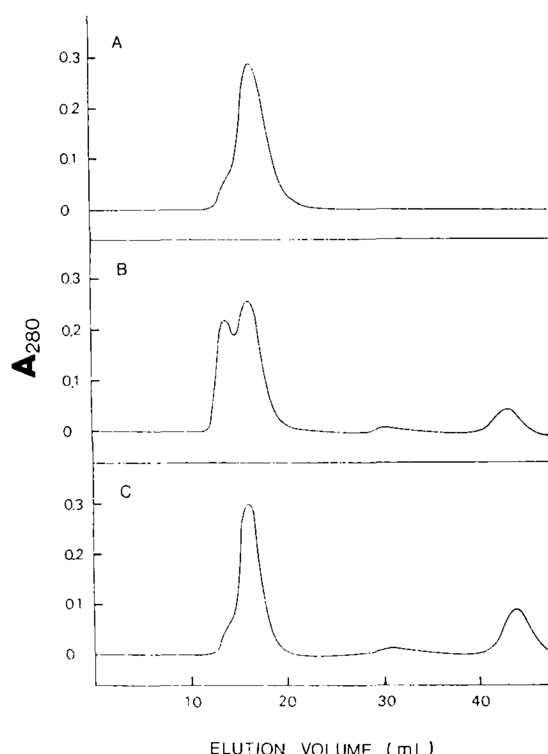

Fig. 1. Gel-permeation chromatography of IgG treated with PMN granules as a source of MPO, H_2O_2 and catechol. Human IgG(4 mg/ml) was treated with PMN granules (40 munits/ml peroxidase), 1 mM H_2O_2 and 1.25 mM catechol in 0.5 ml PBS buffer, pH 7.4 at 37°C in the absence or presence of 2 mM azide. After 1h treatment, 0.2 ml of aliquot was placed on the Sephacryl 200 column. A; untreated IgG, B; IgG treated in the absence of azide and C; IgG treated in the presence of azide. Other details and chromatographic conditions were described in the Materials and Methods.

sodium azide, a inhibitor of MPO, the new peak was not observed and the eluting profile returns to that of normal untreated IgG (Fig. 1.C). Furthermore, omission of one of the three component from the reaction mixture did not give any change to the eluting pattern of normal IgG (data not shown). The observed chromatographic findings indicate that native IgG was polymerized by the reaction medicated by MPO and H_2O_2 in the presence of catechol.

2. Characterization of polymerization of IgG by the MPO-H_2O_2-catechol system

In the previous experiment, polymer formation of IgG by the MPO-H_2O_2-catechol system was confirmed by the gel permeation chromatography. In this experiment, the polymerization by this reaction system was further characterized with direct measurement of the amount of the polymers using the PEG precipitation method. Table 1 shows essential requirement of the three components, MPO, H_2O_2 and catechol for the polymer formation of IgG. With the complete system consisting of the three components, the amount of the polymers formed was 0.48 ± 0.09 mg which corresponds to about 24% of the total IgG added in the reaction mixture. In contrast, no precipitation was observed when native IgG of the same amount with that used in this reaction was mixed with PEG (data not shown). Thus, it was supported that precipited protein with PEG was the IgG polymers. When the reaction was carried out with the omission of any of the components or with complete system in the presence of sodium azide, the amount of the po-

Table 1. Requirements for IgG polymerization*

Reaction conditions	Amount of IgG polymerized** (mg proteins)	Polymerization (%)
Complete system	0.48 ± 0.09	23.9 ± 4.5
+Azide	0.03 ± 0.01	1.5 ± 0.5
−Catechol	0.02 ± 0.01	1.0 ± 0.5
−H_2O_2	0.02 ± 0.02	1.0 ± 1.0
−PMN granules	0.07 ± 0.02	3.8 ± 1.0

* In the complete system, human IgG was treated under the same conditions as in Fig. 1. When indicated, the components shown were added to or omitted from the complete system.

** The polymerized IgG were precipitated with polyethylene glycol and quantitated as described in the Materials and Methods. The results indicate mean ± S.E.M. of 4 to 6 experiments.

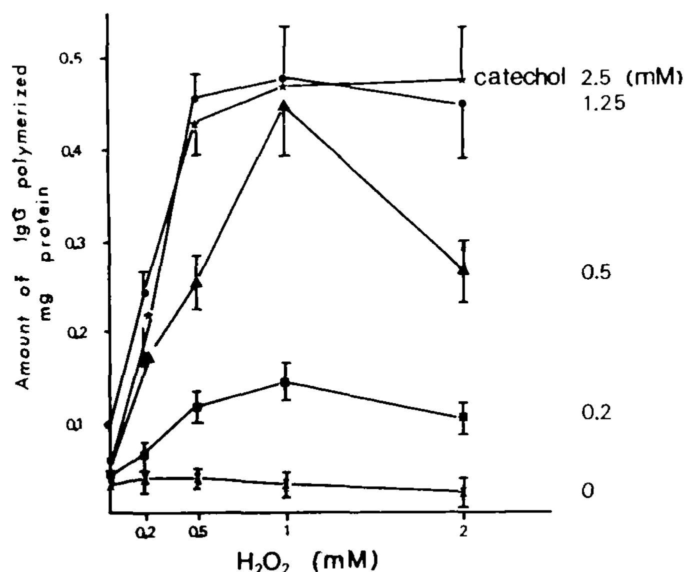

Fig. 2. Concentration effect of H_2O_2 and catechol on the polymerization of IgG. Experimental conditions and determination of polymerized IgG were the same as in Table 1, but with varying concentrations of H_2O_2 and catechol.

lymers was almost negligible. Therefore, the chromatographic finding indicating that the polymer formation of IgG required the three components was also confirmed by the direct assay of IgG polymers.

Fig. 2 shows the effects of concentration of H_2O_2 and catechol on the polymerization of IgG. The polymer formation was increased with increasing concentrations of both H_2O_2 and catechol. In the polymerization of IgG, however, the ratio of H_2O_2 to catechol seems to be important. For example, with 0.5 mM concentration of catechol, the polymerization was increased up to 1 mM H_2O_2, and thereafter, excess H_2O_2 over catechol rather decreased the polymer formation. Under this condition, the decrease in polymerization was recovered again when catechol concentration was increased. The optimal polymerization with the amount of IgG used in this experiment appears to occur with roughly equimolar concentrations of H_2O_2 and catechol.

3. Effects of scavengers for reactive oxygen species and orthoquinones, and antioxidants on the polymerization

Firstly, effects of various scavengers for reactive oxygen species were observed on the polymerization in order to determine whether they were involved in the polymer formation of IgG by the MPO-H_2O_2-catechol. As shown in Table 2, the

Table 2. Effects of various quenchers on polymerization of IgG*

Additions	IgG polymerized (%)
Control	100
+Azide 2 mM	6.3±2.0
+SOD 50 μg/ml	108.6±6.4
+Catalase 50 μg/ml	13.4±3.4
+Mannitol 10 mM	106.0±5.2
+DMSO 10 mM	98.9±6.7
+Histidine 1.2 mM	85.1±8.2
+Benzenesulfinic acid 10 mM	5.0±2.2

* Human IgG was treated with PMN granules, H_2O_2 and catechol under the same conditions as in Fig. 1 but in the presence of the various compounds shown in the table. Control means the amount of polymerized IgG obtained with no compounds added (0.48 ± 0.09 mg protein) which was taken as 100%. Each result shown was mean ± S.E.M. of 4 to 6 experiments.

polymerization was not affected by a O_2^- removing enzyme, SOD, OH• scavengers, DMSO and mannitol, and a singlet oxygen ($^1O_2$) scavenger, histidine. But the polymerization was marked inhibited by catalase decomposing H_2O_2. The observed inhibition by catalase was consistant with that by azide to inhibit MPO mediating peroxidation with H_2O_2 as a substrate. Secondly, effect of benzenesulfinic acid scavenging orthoquinone was observed to elucidate the role of catechol in the polymerization. In contrast to the reactive oxygen species scavengers, the polymerization was inhibited to 95% by this compound (Table 2).

Table 3 shows effects of antioxidants on the polymerization. Among the antioxidants used, ascorbic acid and glutathione inhibited the polymerization. But with the same concentrations as the formers, α-tocopherol and uric acid did not cause any change on the polymerization.

4. Replacement of catechol with various hydrogen donors in the MPO reaction

Catechol used in the present study is not a compound which occurs in the physiological condition. There are various endogenous compounds which are expected to act like catechol serving as a hydrogen donor in the MPO-mediated peroxidation. These compounds were tested if they can substitute catechol in this reaction. The compounds used were epinephrine, dopa, dopamine, ascorbic acid and glutathione (Table 4). Each of which was incu-

Table 3. Effects of antioxidants on the polymerization of IgG*

Additions	IgG polymerized(%)
Control	100
+ α-Tocopherol 1 mM	98.0 ± 8.8
+ Uric acid 1 mM	105.2 ± 8.5
+ Ascorbic acid 0.1 mM	109.4 ± 6.5
+ Ascorbic acid 1 mM	4.5 ± 0.8
+ Gultathione 0.1 mM	103.2 ± 8.1
+ Glutathione 1 mM	17.7 ± 1.7

* Polymerization of IgG by the PMN granules, H_2O_2 and catechol was observed as in Tablè 2, in the presence of various antioxidants shown. The amount of IgG polymerized with no addition of the antioxidant was taken as control.

Table 4. Polymerization of IgG in the presence of various hydrogen donors*

		IgG polymerized (%)
Control		100
Epinephrine	1.25 mM	2.2 ± 0.5
Dopa	1.25 mM	0.7 ± 0.3
Dopamine	1.25 mM	76.5 ± 6.8
Ascorbic acid	1.25 mM	0.7 ± 0.3
Glutathione	1.25 mM	0.3 ± 0.2

* IgG was treated for 1 h with PMN granules and H_2O_2 in the presence of either catechol or other hydrogen donors under the same conditions as in Fig. 1. The amount of IgG polymerized in the presence of catechol was taken as control. Values are mean ± S.E.M. obtained from 4 experiments.

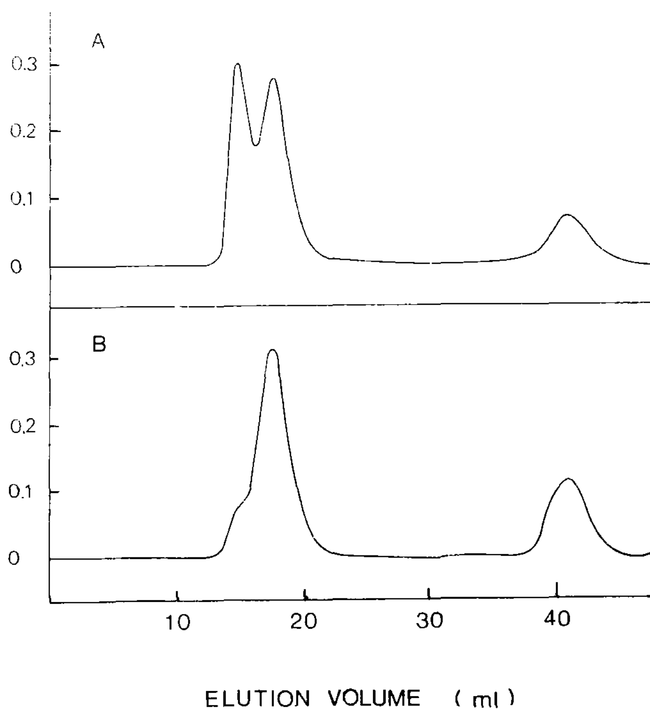

Fig. 3. Gel-permeation chromatography of IgG treated with PMN granules, H_2O_2 and dopamine. IgG was treated with 40 munits/ml PMN granules, 1 mM H_2O_2 and 1.25 mM dopamine in the absence(A) or presence(B) of 2 mM azide as in Table 4. 0.2 ml of aliquot was analyzed on the Sephacryl 200 column as in Fig. 1.

bated with PMN granules, H_2O_2 and IgG under the same condition as in the case when catechol was used. All of them except dopamine were found not to substitute the action of catechol in the polymerization. Only dopamine showed the action of polymerization, which amounted to 76.5% of that of catechol. The action of dopamine was confirmed with gel permeation chromatography, which showed an additional new peak that was the same with that observed in Fig. 1. (Fig. 3).

5. Production of O_2^- and H_2O_2 from human PMNs by IgG polymers.

1) O_2^- production

O_2^- production was estimated as a SOD-inhibitable cytochrome c reduction i.e., a difference of cytochrome C reduction between the absence and presence of SOD in the incubation medium of PMNs and the polymers. As shown in Table 5, a SOD-inhibitable cytochrome C reduction was observed when PMNs were incubated with IgG polymers. The degree of the reduction was dependent on the dose of the polymers. With 320 μg/ml of IgG polymers, the reduction was 1.21 nmoles/10^6 PMNs/15 min. In contrast, untreated IgG of the same amount exhibited one-fourth the reduction obtained with IgG polymers. The findings indicate that IgG polymers formed by the MPO-H_2O_2-catechol system was able to stimulate PMNs more effectively to produce O_2^- Heat-aggregated IgG (320 μg/ml) was also shown to cause the production of O_2^- from PMNs. But its activity was much lower than that of the polymers obtained from the MPO reaction system.

2) H_2O_2 production

H_2O_2 production was measured by fluorscence increase of HVA on its oxidation by horse radish peroxidase in the medium containing PMNs and IgG polymers. H_2O_2 was also produced from PMNs when stimulated by the IgG polymers (Table 6).

Table 5. Production of superoxide anion from human PMNs*

	Cytochrome C reduction nmoles/10^6 PMNs/15 min		
	−SOD	+SOD	Difference
IgG polymers			
40 μg/ml	0.44 ± 0.04	0.21 ± 0.04	0.23 ± 0.04
80 μg/ml	0.71 ± 0.07	0.42 ± 0.10	0.29 ± 0.08
160 μg/ml	1.59 ± 0.26	0.70 ± 0.17	0.89 ± 0.20
320 μg/ml	2.18 ± 0.40	0.79 ± 0.19	1.21 ± 0.31
Heat-aggregated IgG			
320 μg/ml	0.76 ± 0.17	—**	0.76 ± 0.17
Native IgG			
320 μg/ml	0.30 ± 0.03	—**	0.30 ± 0.03

* PMNs were stimulated by various IgGs for 15 min and cytochrome C reduction was determined. Production of superoxide anion from PMNs was expressed as difference in the amount of cytochrome C reduced between in the absence and presence of 50 μg/ml SOD (For the details, see the Materials and Methods). Values are mean ± S.E.M. of 4 experiments.

** Reduction of cytochrome C was negligible.

Table 6. H_2O_2 Production from human PMNs*

	H_2O_2 produced nmoles/10^6 PMNs/30 min
IgG polymers	
40 μg/ml	0.12 ± 0.01
80 μg/ml	0.41 ± 0.04
160 μg/ml	0.68 ± 0.03
320 μg/ml	0.85 ± 0.04
Heat-aggregated IgG	
320 μg/ml	0.63 ± 0.04
Native IgG	
320 μg/ml	0.19 ± 0.01

* Stimulation of PMNs with each of IgGs was performed for 30 min. H_2O_2 production was determined by flurorescence change of HVA (See the Materials and Methods for the details).

The production was dependent on the dose of IgG polymers. When H_2O_2-producing activity of the three IgGs were compared, the IgG polymers of them showed the highest activity. With 320 μg/ml of each, the IgG polymers produced 0.85 nmoles/10^6 PMNs/30 min, untreated IgG, 0.19 nmoles/10^6 PMNs/30 min, heat-aggregated IgG, 0.63 nmoles/10^6 PMNs/30 min.

DISCUSSION

Protein cross-linking has been implicated in a variety of physiologic and pathologic processes, for examples, collagen and elastin fiber maturation (Piez 1968), fibrin clot stabilization, cataract formation (Strivastava and Beutler 1969) and changes in tissue associated with aging process (Leibouitz and Siegel 1980). Recently, oxidative protein cross-lnking was suggested to play a role in toxic reactions to drugs, particularly phenolic or cyclic hydrocarbon derivatives (Jerina and Daly 1974). Stahmann and Spencer (1977) have shown that the peroxidase oxidation products of several hydrogen donors including catechol cause polymerization of some soluble proteins through covalent cross-linking between the protein molecules. In the present study, IgG was chosen as a target protein to be cross-linked using catechol as a hydrogen donor in MPO system of PMNs because the generation of IgG polymers by such oxidative system would have very important pathologic implications with regard to the biochemical mechanisms involved in tissue injury mediated by acute and chronic inflammatory processes.

In this study, it has been clearly shown that IgG polymers were formed by the PMN granules containing MPO and H_2O_2 in the presence of catechol as a hydrogen donor. The three components were essential in the polymerization since the polymer formation was almost negligible by omission of PMN granules, H_2O_2 or catechol, and addition of

catalase or azide to the complete reaction system (Table 1 and 2). In the polymer formation, catechol may serve as an cross-bridge in the form of orthoquinone produced from the peroxidase reaction since the polymerization was significantly inhibited by benzenesulfinic acid, an orthoquinone trap (Table 2). But there is a possibility that the polymerization may result from covalent bond cross-linking between amino acid side chains of IgG which are oxidized by the peroxidase reaction. This possibility does not seem to be probable since involvement of reactive oxygen species which can oxidize the side chains was eliminated in the scavenger study and the polymerization was not influenced by antioxidants such as α-tocopherol and uric acid (Table 3) although other antioxidants, ascorbic acid and glutathione markedly inhibited the polymerization. The inhibitory effect of the latter compounds may be due to the decrease of oxidation of catechol to orthoquinone through the competition of them with catechol in the peroxidase reaction since they can also act as hydrogen donors.

The IgG polymers formed by MPO-H_2O_2-catechol can stimulate PMNs to produce O_2^- and H_2O_2 as the ICs can do and the stimulatory activity was much higher than the heat-aggregated IgG which are known to have biological properties similar to IC_s. The observed stimulatory activity of the polymers may have very important implication in PMN-mediated tissue injury, particularly regarding to the action of MPO system. It is now well established that PMNs release large amount of H_2O_2 and MPO together with other lysosomal enzymes when stimulated by opsonized particles or membrane active agents as well as ICs. This situation can be seen in inflammatory sites where higher amount of MPO and H_2O_2. Although MPO system plays a crucial role in host defense, it also exert a variety of toxic reactions giving harmful effects to surrounding tissues. If there are any suitable hydrogen donors under this condition, IgG could be polymerized and the polymers formed can stimulate PMNs again to produce O_2^- and H_2O_2 probably together MPO and other lysosomal enzymes, all of which can cause tissue damage. Therefore, they can behave like IC_s and can amplify the PMN-mediated tissue injury in a vicious cyclic manner. If this can really happen the cross-linking of proteins may be an additional mechanism for MPO system to mediate tissue injury.

In the present study, polymeriation by MPO system was observed with IgG. But it is also possible that other proteins can also be polymerized by this reaction system. If cross-linking ocurs on cellular proteins, it will ultimately lead to changes of cellular function. This speculation may be supported by the report showing that 6-hydroxydopamine and related compounds cause cross-linking of proteins in cultured neuroblastoma cell, and this phenomenon is related to the cytotoxic mechanism of these substances (Rotmann *et al.,* 1976; Graham *et al.,* 1978). But this possibility remains to be further studied and explored.

The IgG polymers generated by MPO-H_2O_2-catechol was clearly shown to have activity to stimulate PMNs, and possible effects subsequent to this phenomena were discussed above in terms of tissue injury in inflammatory conditions. But some questions should be answered before the potential role of the MPO-induced IgG polymers can be supported. First, although catechol served efficiently in the polymer formation, the compound used as a hydrogen donor is not a physiological compound . Thus, several endogenous compounds were tested to see if catechol can be substituted. Among them tested, only dopamine was able to replace the action of catechol in the MPO-H_2O_2 reaction (Table 4). At present, it is not known whether dopamine really act as a hydrogen donor *in vivo.* But the fact that a physiologically occurring compound can make the IgG polymers as effectively as catechol suggests a possibility of polymerization of IgG by MPO-H_2O_2 system *in vivo.* Jasin (1983) proposed that a quinone-like material, ubiquinone-50 could be a candidate which can act as catechol. But in addition to dopamine or ubiquinone, physiologically acting compounds should be sought through testing many other endogenous candidate compound. Second, the polymers should be detected in body fluid. The polymers obtained in the present study were not characterized at molecular level. The detailed properties such as molecular size and cross-linking points should be explored for the detection of IgG polymers *in vivo.*

In conclusion, IgG polymers can be formed by MPO-H_2O_2 reaction in the presence of hydrogen donors, for example, catechol. The polymers can stimulate PMNs as IC_s do. This implies that the polymers play a potential role in PMN-induced tissue injury in inflammatory conditions and serves as an additional mechanism to amplify the adverse effects of MPO-mediated peroxidation.

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= 국문초록 =

Myeloperoxidase-H_2O_2-Catechol계에 의한 인체 면역글로불린 G의 polymer 형성과 이들의 백혈구 산소라디칼 생성 촉진 효과

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인체 면역글로불린G (IgG)를 hydrogen donor로 작용하는 catechol 존재하에서 myeloperoxidase(MPO)의 공급원으로서 다형핵 백혈구 (PMN) 과립 및 H_2O_2와 처리한 후 Sephacryl 200 column chromatography 및 polyethylene glycol 침전법을 통하여 분석한 결과, 이 단백질이 polymer로 변화됨을 확인하였다.

이와같은 polymer 형성은 IgG를 PMN 과립, H_2O_2 혹은 catechol중 어느 한 가지를 빼고 반응시키거나 세요소가 모두 포함된 조건에서 catalase 혹은 MPO 억제제인 azide를 첨가하여 반응시켰을 경우에는 전혀 관찰되지 아니하였다. MPO-H_2O_2-catechol계에 의한 IgG의 polymer 형성은 여러 산소대사물 제거제 (SOD, DMSO 및 histidine)와 항산화제 (α-tocopherol 및 uric acid)에 의해서는 영향을 받지 아니하였으나 orthoquinone 제거제인 benzenesulfinic acid에 의해서 억제되었다. 따라서 이 반응계에 의한 IgG polymer 형성에는 산소대사물이 관여되지 않고 MPO와 H_2O_2에 의한 catechol 산화물인 orthoquinone이 중요한 역활을 함을 알 수 있었다.

Hydrogen donor로 작용할 가능성이 있는 수종의 내인성 물질 (epinephrine, dopa, dopamine, ascorbic acid, glutathione)을 catechol 대신 사용한 결과 사용된 물질 중 dopamine만이 유의하게 polymer을 형성시켰다 (catechol 사용시의 76.5%).

한편 MPO-H_2O_2-catechol계에 의하여 얻어진 IgG polymer는 인체 PMN을 자극하여 O_2^-와 H_2O_2를 생성시키는 효과를 나타내었다. 이와같은 산소라디칼 생성 촉진 효과는 처리하지 않는 IgG의 경우 아주 미약하여 IgG polymer의 1/4 정도의 효과를 보였다. Heat-aggregated IgG도 산소라디칼 생성 촉진효과를 나타냈으나 IgG polymer의 70% 정도에 지나지 아니하였다.

이상의 실험결과를 통하여 IgG는 MPO 산화계에 의하여 polymer로 변화될 수 있고 이 polymer는 항원-항체 복합체와 같이 PMN을 자극할 수 있는 성질이 있음을 알수 있었다. IgG polymer의 이와같은 성질이 급만성 염증에서 조직손상에 관여할 가능성에 대하여 고찰하였다.