ACTIVATION OF FACTOR XII BY TOBACCO GLYCOPROTEIN*

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Tobacco glycoprotein (TGP)[1] is a brown, iron-containing substance of mol wt ca. 18,000 isolated from saline extracts of flue cured tobacco leaves by ammonium sulfate fractionation, chromatography on Sephadex G-25, and continuous flow, preparative electrophoresis on alkaline polyacrylamide gel. 12 of 31 volunteers (6/15 smokers and 6/16 nonsmokers) exhibited immediate cutaneous hypersensitivity reactions characterized by wheal and flare development and local itching when injected intracutaneously with this material. Immunochemically similar material was demonstrated in, and isolated from, cigarette smoke. Antigenically cross-reactive material was also demonstrated in eggplants, green peppers, potatoes, and tomatoes, which like tobacco, are members of the family *Solanaceae* (1).

The brown color, molecular weight, amino acid analysis, iron content, and presence of carbohydrate moiety in TGP correspond to that of an iron-protein-chlorogenic acid-rutin complex in cured tobacco leaves first reported by Wright et al. (2). Since rutin is chemically similar to quercetin, a substance known to activate factor XII (3), we examined whether TGP isolated from cured Virginia Bright tobacco leaves or from cigarette smoke condensate was capable of activating factor XII in human blood plasma. In experiments described below we demonstrated that TGP derived from cured tobacco leaves or from cigarette smoke condensate contained rutin or a rutin-like substance and activated factor XII in samples of human plasma resulting in the generation of clotting activity, fibrinolytic activity, and kinin activity.

Materials and Methods

Tobacco Leaves and Cigarette Smoke Condensate. Cured tobacco leaves of Virginia Bright variety were supplied by the American Tobacco Co. and by the Phillip Morris Co. through the courtesy of Dr. Robert C. Hockett of The Council for Tobacco Research-U. S. A., Inc., New York. Cigarette smoke condensate, produced from University of Kentucky 1R1 cigarettes smoked in a standard manner was, also courtesy of Dr. Hockett, kindly supplied by Dr. M. R. Guerin of Oak Ridge National Laboratories, Oak Ridge, Tenn.

Isolation of TGP. TGP was isolated from these sources essentially according to methods

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[1] *Abbreviations used in this paper:* BSA, bovine serum albumin; PBS, phosphate-buffered physiologic saline, pH 7.4; PTT, partial thromboplastin time; TGP, tobacco glycoprotein; TGP-CSC, TGP derived from cigarette smoke condensate; TGP-L, TGP derived from cured tobacco leaves.

previously described, except that Bio/Gel P-6 (Bio-Rad Laboratories, Richmond, Calif.) was used instead of Sephadex-25 (1). TGP derived from cured tobacco leaves will be referred to as TGP-L. TGP derived from cigarette smoke condensate will be referred to as TGP-CSC. Both TGP-L and TGP-CSC were present as a single band when electrophoresed on alkaline polyacrylamide gels, as described previously (1).

Rabbit Antiserum to TGP. Rabbit antibodies to TGP were prepared as described previously (1).

Rutin-Bovine Serum Albumin Conjugate and Preparation of Rabbit Antiserum Thereto. 200 mg of rutin (Sigma Chemical Co., St. Louis, Mo.) was suspended in 2 ml of phosphate-buffered physiologic saline, pH 7.4 (PBS), in a glass beaker and the pH brought to 11.2–11.5 with 1 N NaOH. This was placed in an ice bath and 0.5 g of CNBr dissolved in 2.5 ml of dimethylformamide was added dropwise. pH was maintained at 10.5–11.0 by addition of 5 N NaOH with stirring for 7–8 min. 10 mg of crystalline bovine serum albumin (BSA) (Sigma Chemical Co.) was added and the mixture stirred at room temperature for 2 h and overnight at 4°C. The mixture was then dialyzed for 2 days against three changes of PBS and then concentrated by pressure dialysis using an Amicon PM-10 filter (Amicon Corp., Scientific Systems Div., Lexington, Mass.) and applied to a 2.5 x 50 cm column of Bio/Gel P-6 equilibrated with PBS. The brown rutin-BSA conjugate emerged with the void volume; unconjugated rutin emerged later as a second peak. Rutin-BSA was then dialyzed exhaustively against Pyrex distilled water and lyophilized. The yellow-brown powder was stored for use at −20°C. Three Dutch-belted rabbits were each inoculated intracutaneously in two sites with 200 μg of rutin-BSA in complete Freund's adjuvant. They were boostered 3 wk later and bled after 2 more weeks. The serum was collected from the clotted blood and stored at −70°C.

Hemagglutination Inhibition Assays. Washed human erythrocytes of blood group O were tanned and coated with TGP according to methods described by Herbert (4). Rabbit antisera to TGP, rabbit antisera to rutin-BSA, and pooled normal rabbit serum were heat inactivated and adsorbed with washed packed type O erythrocytes. Serum dilutions were prepared in Microtiter U-plates (Cooke Laboratory Products Div., Dynatech Laboratories Inc., Alexandria, Va.). The titer of anti-TGP antibodies ranged between 1:256 and 1:1,024. Serum from two of the rabbits immunized with rutin-BSA agglutinated TGP-coated erythrocytes in titers of 1:512 and 1:1,024. Pooled normal rabbit serum contained no antibodies to TGP.

Hemagglutination inhibition assays were performed according to methods described by Kwapinski (5). The capacity of ellagic acid (K&K Laboratories Inc., Plainview, N. Y.), quercetin (Sigma Chemical Co.), rutin, TGP-L, TGP-CSC, BSA, or PBS to inhibit agglutination of TGP-coated erythrocytes by rabbit anti-TGP-L or by rabbit anti-rutin-BSA sera was measured. As a control for nonspecific inhibition of agglutination, tanned human type O erythrocytes were coated with human IgG purified by DEAE-cellulose chromatography and a hemagglutination system was constructed using heavy chain-specific rabbit antisera to human IgG (Behring Diagnostics, American Hoechst Corp., Woodbury, N. Y.). The capacity of human IgG, ellagic acid, quercetin, rutin, TGP-L, TGP-CSC, or PBS to inhibit agglutination of human IgG-coated erythrocytes by rabbit antiserum to IgG was measured. The quantity of TGP-L or TGP-CSC in solution was determined by the Lowry et al. (6) modification of the technique of Folin and Ciocalteau, using a TGP standard as described previously (1). The quantity of BSA or IgG in solution was also determined by the Lowry method using a BSA standard. The quantity of the other substances was determined gravimetrically.

Human Plasma. Normal human venous blood was obtained from laboratory personnel and anti-coagulated with sodium citrate (7). Some donors had previously exhibited immediate cutaneous hypersensitivity to TGP and some had not (1). Platelets and other formed elements were removed by centrifugation in plastic tubes at 4°C and plasma was pipetted with plastic pipette tips into plastic tubes and either placed in crushed ice before immediate use or stored at −70°C. Human plasma deficient in factor XII was obtained from George King Bio-Medical, Inc., Salem, N. H., and stored at −70°C.

Partial Thromboplastin Time. A fibrometer (Baltimore Biological Laboratories, Baltimore, Md.), plastic pipette tips, plastic tubes, and the following reagents were used: Celite (Filter Aid; Fisher Scientific Co., Pittsburgh, Pa.) in PBS in a concentration of 5 mg/ml, 0.05M $CaCl_2$ in glyoxaline buffer (7), and rabbit brain cephalin (Sigma Chemical Co.) in PBS. In these experiments, 0.1 ml of plasma was pipetted in a plastic reaction vessel and warmed at 37°C for 60 s. 0.1 ml of Celite suspension was then pipetted in and the mixture warmed for 60 s. 0.05 ml of cephalin

solution and 0.05 ml of $CaCl_2$ solution were then added and the timer and probes started. The timer stops automatically when a clot forms. The partial thromboplastin time (PTT) of normal plasma activated with Celite ranged between 75 and 100 s. When PBS was used instead of Celite the PTT was between 400 and 600 s depending on whether the plasma had been frozen at −70°C or was fresh. The following substances, dissolved in PBS, were tested for their capacity to replace Celite in this assay: TGP-L, TGP-CSC, ellagic acid, quercetin, rutin, rutin-BSA conjugate, BSA, ragweed antigen E, beta lactoglobulin, and PBS. All values represent the mean of duplicate determinations. The coefficient of variation in PTT by this technique was 6%.

Euglobulin Clot Lysis Time. The capacity of ellagic acid, quercetin, rutin, rutin-BSA, BSA, TGP-L, TGP-CSC, and Celite to activate fibrinolysis was measured essentially according to the technique of Ogston et al. (8).

Citrated human plasma (0.5 ml) was incubated in plastic tubes with 0.9 ml of Celite suspension, PBS, or test substances for 60 min at 37°C. This mixture was then diluted with 11.5 ml of cold 0.01 M sodium acetate buffer, pH 4.8, and euglobulin precipitated at 4°C for 30 min. The tubes were centrifuged at 5,000 *g* at 4°C, the supernate decanted, and the precipitate dissolved in 0.5 ml of barbital saline buffer, pH 8.4 and transferred to 10 x 75 mm Pyrex tubes. 0.1 ml of bovine thrombin (Thrombin Topical; Parke, Davis & Co., Detroit, Mich.) in PBS at a concentration of 20 U/ml was then squirted in so that a clot formed with bubbles. The tubes were then transferred to a water bath at 37°C. The clot lysis time was taken as the interval between the addition of thrombin and the lysis of the clot as indicated by the rise of trapped bubbles. All determinations were performed in duplicate. Values shown represent the mean of duplicate determinations. The concentrations of TGP-L and TGP-CSC used were those which resulted in maximal shortening of the partial thromboplastin time.

Kinin Generation. 0.2 ml of plasma was warmed for 1 min at 30°C. 0.2 ml of 0.01 M disodium EDTA and 0.2 ml of the test substance at a concentration in PBS known to shorten the partial thromboplastin time was then added and the mixture incubated at 37°C for 3 min. 0.2 ml of a solution of soybean trypsin inhibitor (Worthington Biochemical Corp., Freehold, N. J.) in PBS (4 mg/ml) was then added. 0.4 ml of this mixture was then injected into a 10 ml De Jalon's buffer bath containing the uterine horn of a 150 g Sprague-Dawley rat in natural estrus. Contraction was measured isotonically with a linear motion transducer, model ST-2, the transformer of which was supplied by an exciter demodulator (Phipps & Bird Inc., Richmond, Va.) and recorded on a Bausch and Lomb VOM-5 recorder, as described by Harpel (9). Contractions were compared to those induced by standard dilutions of bradykinin triacetate (Sigma Chemical Co.) in De Jalon's buffer. The amount of kinin generated from plasma is expressed in terms of the bradykinin concentration necessary to induce comparable uterine contractions. Factor XII fragments were kindly provided by Dr. Peter C. Harpel (9).

Test for Endotoxin. Solutions of ellagic acid, quercetin, rutin, TGP-L, TGP-CSC, and PBS used as diluent were tested for bacterial endotoxin using the Limulus amebocyte lysate test (10). Reagents for this assay were obtained from Microbiological Associates, Bethesda, Md. Bacterial endotoxin was not demonstrable by this method in these solutions.

Results

Hemagglutination Inhibition Assay. It can be seen in Table I that quercetin, rutin, TGP-L, and TGP-CSC can inhibit agglutination of TGP-L-coated human type O erythrocytes by rabbit antiserum to rutin-BSA conjugate. This indicates that quercetin or rutin moieties are present in molecules of TGP-L and TGP-CSC and are arranged in such a way that they are immunologically active. The fact that TGP-CSC inhibits agglutination less well than TGP-L indicates that it contains less of this moiety or that it has been altered during smoking.

Ellagic acid, quercetin, rutin, TGP-L, and TGP-CSC inhibited agglutination of human type O erythrocytes coated with TGP-L by rabbit antiserum to TGP-L (Table II). These observations indicate that moieties resembling ellagic acid, quercetin, or rutin are present in TGP and act as haptens to stimulate production of specific antibody. These data also support the concept that these moieties

TABLE I

Hemagglutination Inhibition Assay Comparing TGP-L, TGP-CSC, Rutin, Quercetin, and Ellagic Acid, Using Rabbit Antibody to Rutin-BSA

Antigen	Rabbit serum*	Cell label	Agglutination	Minimum inhibiting antigen concentration
TGP-L	Anti-rutin-BSA	TGP-L	–	125–250 ng/ml
TGP-CSC	Anti-rutin-BSA	TGP-L	–	2.45–4.9 μg/ml
Rutin	Anti-rutin-BSA	TGP-L	–	1 μg/ml
Quercetin	Anti-rutin-BSA	TGP-L	–	10 μg/ml
Ellagic acid	Anti-rutin-BSA	TGP-L	+	(No inhibition)
BSA	Anti-rutin-BSA	TGP-L	+	(No inhibition)
PBS	Anti-rutin-BSA	TGP-L	+	(No inhibition)
PBS	Anti-rutin-BSA	Unlabeled	–	
PBS	Normal	TGP-L	–	
PBS	Normal	Unlabeled	–	

* Serum was diluted 1:128.

TABLE II

Hemagglutination Inhibition Assay Comparing TGP-L, TGP-CSC, Rutin, Quercetin, and Ellagic Acid, Using Rabbit Antibody to TGP-L

Antigen	Rabbit serum*	Cell label	Agglutination	Minimum inhibiting antigen concentration
				μg/ml
TGP-L	Anti-TGP-L	TGP-L	–	5.9
TGP-CSC	Anti-TGP-L	TGP-L	–	9.1
Rutin	Anti-TGP-L	TGP-L	–	16
Quercetin	Anti-TGP-L	TGP-L	–	16
Ellagic acid	Anti-TGP-L	TGP-L	–	40
BSA	Anti-TGP-L	TGP-L	+	(No inhibition)
PBS	Anti-TGP-L	TGP-L	+	(No inhibition)
PBS	Anti-TGP-L	Unlabeled	–	
PBS	Normal	TGP-L	–	
PBS	Normal	Unlabeled	–	

* Serum was diluted 1:360.

on TGP are accessible to macromolecules. Neither ellagic acid, quercetin, rutin, TGP-L, nor TGP-CSC inhibited agglutination by rabbit antiserum to human IgG of erythrocytes coated with human IgG, although human IgG in quantities as low as 100 ng/ml inhibited this reaction.

Effect of TGP-L and TGP-CSC on PTT. The effect of TGP-L and TGP-CSC on the PTT of normal plasma was biphasic (Table III). Certain concentrations of either TGP-L or TGP-CSC shortened the PTT of normal plasma, whereas higher concentrations prolonged the PTT. The effect was the same in plasma irrespective of whether or not donors exhibited immediate cutaneous hypersensitivity to

TABLE III
Effect of Different Concentrations of TGP-L and TGP-CSC on PTT of Normal Plasma

Substance	Concentration in reaction mixture	Clotting time
	μg/ml	*s*
TGP-L	400	685
TGP-L	200	425
TGP-L	100	350
TGP-L	50	400
TGP-L	25	450
TGP-L	12.5	600
Celite	1.66 mg/ml	83
PBS		550
TGP-CSC	250	729
TGP-CSC	125	435
TGP-CSC	63	375
TGP-CSC	32	275
TGP-CSC	16	435
TGP-CSC	8	525
TGP-CSC	3	560
Celite	1.66 mg/ml	85
PBS		570

TABLE IV
Effect of Ellagic Acid, Quercetin, Rutin, TGP-L, TGP-CSC, and Rutin-BSA on PTT of Normal Plasma and Plasma Deficient in Factor XII

Substance	Concentration in reaction mixture	Clotting time (normal plasma)	Clotting time (factor XII-deficient plasma)
	μg/ml	*s*	*s*
Ellagic acid	27	180	>1,200
Quercetin	41	140.8	>1,200
Rutin	41	173.3	>1,200
TGP-L	100	350	>1,200
TGP-CSC	32	275	>1,200
Rutin-BSA	83	280	>1,200
BSA	83	600	>1,200
Celite	1.66 mg/ml	83	>1,200
PBS		550	>1,200

TGP-L. Concentrations of TGP-L and TGP-CSC which were optimal in shortening the PTT of normal plasma did not shorten the PTT of plasma deficient in factor XII (Table IV). These observations indicate that TGP-L or TGP-CSC can activate factor XII, presumably because of quercetin or rutin moieties.

To test this hypothesis we compared the effect of different concentrations of rutin-BSA conjugate on the PTT of normal plasma. The effect was similar to that produced by TGP-L or TGP-CSC (Table V). High concentrations shortened the PTT of normal plasma. Comparable concentrations of BSA had no effect on the PTT. Concentrations of rutin-BSA which shortened the PTT of normal

TABLE V
Effect of Different Concentrations of Rutin-BSA and of BSA on PTT of Normal Plasma

Substance	Concentration in reaction mixture	Clotting time
	μg/ml	*s*
Rutin-BSA	330	>1,200
Rutin-BSA	167	494
Rutin-BSA	83	280
Rutin-BSA	42	283
Rutin-BSA	21	278
Rutin-BSA	5.2	369
Rutin-BSA	1.3	483
Rutin-BSA	0.65	608
Celite	1.66 mg/ml	93
PBS		643
BSA	330	600
BSA	83	647.3
BSA	21	635
Celite	1.66 mg/ml	92.8
PBS		621

TABLE VI
Effect of TGP-L or TGP-CSC on Thrombin Time of Normal Plasma

Substance	Concentration in reaction mixture	Thrombin time
	μg/ml	*s*
TGP-L	400	22
TGP-CSC	400	21
PBS		21

plasma had no effect on the PTT of plasma deficient in factor XII (Table IV). These observations indicate that the attachment of rutin or quercetin-like moieties to protein converts normally inactive molecules to activators of factor XII.

To see if the prolongation of PTT by high concentrations of TGP-L, TGP-CSC, or rutin-BSA was due to a heparin-like effect, the effect of high concentrations of these substances on the thrombin time was measured in comparison to the PBS diluent control. These substances had no heparin-like effect on the thrombin time (Table VI). These studies suggest the capacity in high concentrations of TGP-L, TGP-CSC, or rutin-BSA to prolong the PTT of normal plasma is probably due to complex formation with factor XII or other components of the first phase of the intrinsic blood coagulation system.

In other experiments we examined the possibility of whether other purified allergens, such as antigen E of ragweed or beta lactoglobulin of cow's milk, might also effect the PTT of normal plasma. These allergens had no effect.

Effect of TGP-L, TGP-CSC, and Rutin-BSA on Activation of Fibrinolysis. Activation of factor XII by Celite, kaolin, or ellagic acid is known to result in activation of the fibrinolytic system of normal plasma (8, 11, 12). Therefore, if

TABLE VII
Effect of TGP-L, TGP-CSC, Rutin, and Rutin-BSA on the Generation of Fibrinolytic Activity from Normal Plasma and from Factor XII-Deficient Plasma

Substance	Final concentration in reaction mixture	Clot lysis time	
		Normal plasma	Factor XII-deficient plasma
	μg/ml	*s*	*h*
TGP-L	100	345	>6
TGP-CSC	32	375	>6
Rutin	41	360	>6
Rutin-BSA	83	390	>6
BSA	83	840	>6
Celite	1.66 mg/ml	315	>6
PBS		870	>6

TGP-L, TGP-CSC, or rutin-BSA shortened the PTT by activating factor XII as these data suggest, then activation of the fibrinolytic system should also be demonstrable in normal plasma treated with these substances. Concentrations of TGP-L, TGP-CSC, rutin, or rutin-BSA that shorten PTT of normal plasma, like Celite, also shorten the lysis time of euglobulin clots (Table VII). The lysis time was not shortened by BSA or PBS diluent, nor was the euglobulin clot lysis time of factor XII-deficient plasma shortened by these substances. These data provide further evidence that TGP-L or TGP-CSC activate factor XII because of quercetin or rutin-like moieties.

Generation of Kinin by TGP-L, TGP-CSC, Rutin, and Rutin-BSA. In addition to activating the intrinsic blood coagulation system and the fibrinolytic pathway of normal plasma, activation of factor XII is known to result in the generation of bradykinin (12, 13).

Treatment of normal plasma with concentrations of TGP-L, TGP-CSC, rutin, or rutin-BSA conjugate, that resulted in shortening of PTT and euglobulin clot lysis time also resulted in generation of kinin activity as measured by the capacity of plasma to stimulate contraction of the isolated uterine horn of the estrus rat (Table VIII). Treatment of normal plasma with ellagic acid, a known activator of factor XII, also resulted in generation of kinin activity. Neither BSA nor PBS diluent initiated kinin generation in normal plasma. None of the activator substances initiated kinin generation in factor XII-deficient plasma. When factor XII fragments were added to factor XII-deficient plasma kinin was generated, indicating that with the exception of factor XII, the kinin generation system was otherwise intact.

Discussion

Glycoprotein isolated from flue cured Virginia Bright tobacco leaves (TGP-L) and from cigarette smoke condensate (TGP-CSC) (*a*) shortened the PTT of normal plasma; (*b*) activated the fibrinolytic system of normal plasma; and (*c*) activated the generation of kinin from normal plasma. These effects were not demonstrable in plasma from a patient deficient in factor XII (Hageman factor).

TABLE VIII

The Generation of Kinin Activity by Ellagic Acid, Rutin, TGP-L, TGP-CSC, and Rutin-BSA from Normal Plasma and from Factor XII-Deficient Plasma as Measured by Bioassay

Substance	Normal plasma (ng of bradykinin/0.1 ml of plasma)	Factor XII-deficient plasma (ng of bradykinin/0.1 ml of plasma)
Ellagic acid	>20	0
Rutin	5	0
TGP-L	15	0
TGP-CSC	5	0
Rutin-BSA	>20	0
BSA	0	0
PBS	0	0

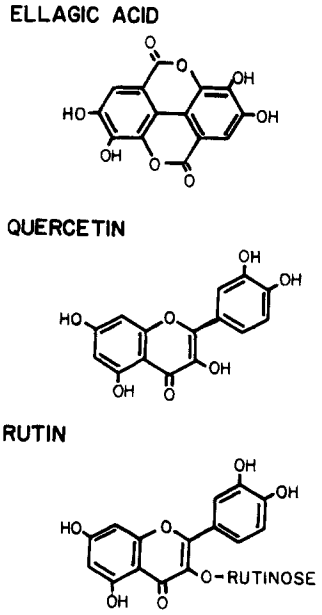

FIG. 1. Comparison of structures of ellagic acid, quercetin, and rutin.

Activation of factor XII is known to result in acceleration of the PTT, activation of the fibrinolytic system, and the generation of bradykinin (12). Thus, these data indicate that TGP-L and TGP-CSC are capable of activating factor XII in normal plasma. Although TGP may directly activate factor XII, it is possible that this is accomplished indirectly through activation of high molecular weight kininogen (14).

The capacity of TGP to activate factor XII is due, apparently, to the presence of rutin. Rutin closely resembles quercetin and partially resembles ellagic acid (Fig. 1). Quercetin and ellagic acid both are known to activate factor XII (3). In experiments described here, rabbit antibodies to rutin-BSA conjugates agglutinated tanned human type O erythrocytes coated with TGP-L. This agglutination was inhibited with quercetin, rutin, TGP-L, or TGP-CSC, indicating that rutin

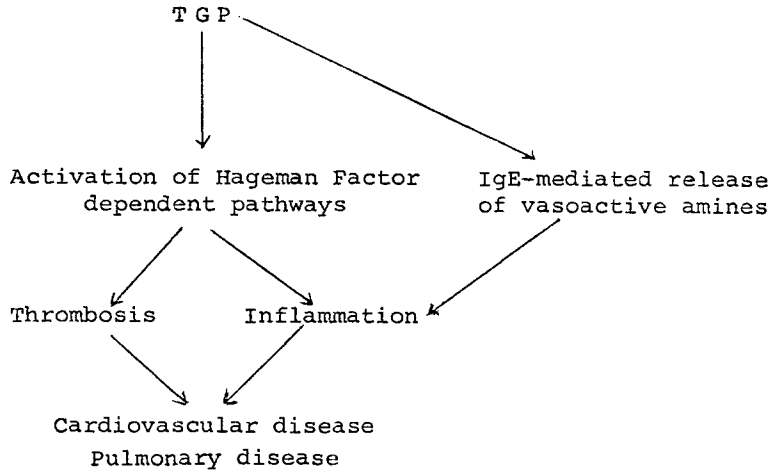

FIG. 2. Hypothesis concerning the relationship of TGP to the pathogenesis of pulmonary and cardiovascular disease.

was not only present in molecules of TGP-L or TGP-CSC, but that it was immunologically reactive. TGP-L was a more potent inhibitor (Table I) of agglutination than TGP-CSC, indicating a higher content of rutin per molecule or differences in the arrangement of rutin on the molecule, possibly as a consequence of the smoking process. In similar experiments, rabbit antibodies to TGP-L agglutinated human type O erythrocytes coated with TGP-L. Agglutination was inhibitable with ellagic acid, quercetin, rutin, TGP-L, and TGP-CSC. This experiment provides further evidence for the presence of rutin, or material resembling rutin, in TGP and indicates that substances with this structure can act as haptens in stimulating antibody production to TGP. Our study also demonstrates that attachment of rutin to an otherwise inert carrier molecule, such as BSA, can cause the carrier to become an activator of factor XII.

The question arises as to whether the separate phenomena of capacity to activate factor XII and immunogenicity are related functionally. It is conceivable that structures capable of activating factor XII are especially potent immunogens. It is known that activated factor XII stimulates macrophages to spread (15) and that macrophages are important to T-cell-B-cell collaboration in antibody production (16). The high incidence of immediate cutaneous hypersensitivity to TGP-L may be a function of these interrelationships acting in concert with antigenic ubiquity, since antigens cross-reactive with TGP have been identified in extracts of other common *Solanaceae* such as eggplants, green peppers, potatoes, and tomatoes.

The fact that TGP can activate factor XII and is a potent allergen may be the basis of the relationship between cigarette smoking, pulmonary disease, and cardiovascular disease (17–21). A working hypothesis is outlined in Fig. 2. Inhalation of cigarette smoke could result in the chronic activation of factor XII and the generation of mediators of the inflammatory response by TGP, resulting in destruction of pulmonary parenchyma. Immunologically mediated release of vasoactive amines in response to TGP may act synergistically with this mechanism in the progression of pulmonary disease (1, 22). When inhaled the relatively small size of TGP (ca. 18,000 daltons) may permit easy access to the

circulating blood. Activation of factor XII by TGP and initiation of thrombosis or kinin generation could result in increased endothelial permeability or focal vascular injury. These changes may be augmented by the immunologically induced release of vasoactive amines in the cigarette smoker hypersensitive to TGP (1, 22).

Summary

A glycoprotein of mol wt ca. 18,000 daltons isolated from cured tobacco leaves (TGP-L) and from cigarette smoke condensate (TGP-CSC) activated factor XII in normal human plasma in vitro as measured by (*a*) shortening of the partial thromboplastin time, (*b*) shortening of the lysis time of euglobulin clots, and (*c*) generation of kinin activity. These effects were not demonstrable in plasma deficient in factor XII. The capacity of TGP-L and TGP-CSC to activate factor XII was shown to depend on the presence of rutin, a substance chemically similar to quercetin and ellagic acid, which are known activators of factor XII. Rutin and rutin coupled to bovine serum albumin, but not bovine serum albumin alone, were also demonstrated to activate factor XII. The presence in cigarette smoke of material that is both allergenic and capable of activating factor XII of the intrinsic pathway of coagulation may be important to the pathogenesis of cardiovascular and pulmonary disease associated with cigarette smoking.

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