ANTIGENIC UNITY BETWEEN PHYTOHEMAGGLUTININ OF PHASEOLUS VULGARIS AND SOME BACTERIA AND VIRA

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The immunologic essence of the proliferation and blastic transformation of lymphocytes under the effect of phytohemagglutinin (PHA) and a variety of other antigens (bacterial, viral and tissue) has been demonstrated by many authors (10, 18, 20, 21, 23 etc.). Yet, there is discordance on the mechanism of this immunologic reaction — whether the latter is of the type of secondary immune response or else, it has a different nature are questions still awaiting solution (8, 15, 16, 17, 24).

The arguments set forward by various investigators, though well-grounded, are not sufficient to discard the idea that in the action of PHA exerted upon lymphocytes, regularities are manifested inherent to the secondary immune reaction. The fact that the mitogenic effect of one and the same PHA displays differences in single individuals, being even negative in some cases, also supports such an assumption. It is believed that such an individual reactivity is related to the immune state of different individuals and is conditioned by genetic and external factors. For instance, it has been established that tuberculin-positive individuals react to the effect of PHA much more strongly as compared to tuberculin-negative cases (23). The leukocytes of persons with recent infectious or allergic illnesses in the past history are manifested as hyporeactive (19) or areactive towards the drugs and other substances received, with destructive changes being occasionally observed under the effect of the bacterial or some other antigen or allergen (7, 12 etc.). The lymphocytic destruction was also noted during the application of large concentrations PHA in leukocyte culture, in vitro (Tzoneva-Maneva). It has been pointed out moreover, that there are differences in the degree of natural precipitation of the blood sera of various individuals under the effect of PHA (Tzoneva-Maneva). Possibly, the stated quantitative and qualitative difference is the expression of their individual humoral immune activity.

Bacterial phytohemagglutinins have been discovered as early as 1902 (15) in staphylococci, vibrions whilst other authors described them also in Haemophylus Pertussis, Escherichia coli etc. (25).

Well known are likewise the hemagglutinins in the various vira — a circumstance which is utilized in the Hirst reaction in the course of routine diagnostics (9). Tobiska (25) is the first to pose the question about the eventual unity between PHA and hemagglutinins of viral origin after establishing that both types hemagglutinins are being inhibited by the same substances — ovalbumin, saliva, pectines and others. The same writer reports a fall in the agglutination of rabbit erythrocytes towards PHA from beans after beforehand treatment with grippe virus PR-8 and NDV. Similar are also the data reported by Bourdillon (6). The latter fact is an

indirect proof for the identity of some of the PHA globulin receptors with those of the vira studied.

The observations of Hirschhorn and assoc. (13) also support the manifestation of immuno-specific mechanisms in the mitogenic action of PHA; the same authors established the absence of proliferation and de-differentiation within the lymphocytes of sarcoidosis patients under the effect of PHA and tuberculin and a positive reaction under the effect of the Kweim-antigen, used as an immunological test in sarcoidosis.

Similar have been also the reactions of patients with various lymphoproliferative diseases. A correlation between immunomorphological response and the hypergammaglobulinemia was also noted in such cases (13). Forbes (11) discovers numerous mitoses within the peritoneal liquid of rodents after the administration of the endotoxin Escherichia coli.

The facts herein described render our conjecture scientifically substantiated, insofar mitogenic activity of PHA might be due to a unity between antigenic PHA components and some bacterial and viral components.

In support of our hypothesis are also the data reported by Bird (5), who, under special conditions (Immune Precipitation Serum dilution — IPS), established an antigenic unity between PHA of Ricinus communis and Abrus precatorius and pneumococcus type XIV.

The chief goal of the present work is the clarification of the questions thus outlined.

Material and Method

In the experiments the methods of double diffusion in gel according to Ouchterlony (22) and microimmunoelectophoresis after Grabar and Bourtin (1) were employed.

The immune precipitation sera (IPS) anti-PHA were obtained through immunization of rabbits with PHA from Phaseolus vulgaris, sort Sax.

By the method of double gel diffusion we set an experiment for the detection and identification of eventually common PHA antigens and antigens from bio-products of a variety of infectious agents: tuberculin, vaccinal virus, tetanic anatoxin, dyphtheria-tetanus-pertussis, rabies vaccine, bacteriophage, attenuated poliomyelitis vaccine, typhoid vaccine, dysentery-typhoid vaccine, typho-paratyphoid vaccine, grippe virus A-2 and Staphylococcus aureus. The experimental setting was repeatedly carried out (twice).

Toxic Staphylococcus aureus strain, Streptococcus β hemolyticus and grippe virus A-2 were used during the microimmunoelectrophoresis.

As antigens were applied: 1) Staphylococcus aureus toxin, obtained through filtration of 4-day culture, grown on ordinary agar, through shot-filter G-5; 2) polysaccharide extract, obtained from 24-hour culture of Streptococcus β haemolyticus, cultivated over pyruvate broth, with subsequent sedimentation and autoclave sterilization of the sediment, diluted in physiological saline solution (we used the top fluid part overneath the sedimentation level (4); 3) from the grippe virus A-2, we employed its soluble antigen in the allantoic fluid of contaminated hen embryo (3,2).

Double (6 gr %) PHA concentration was employed in physiological saline solution as compared to that, utilized in the first experimental setting — investigations by double gel diffusion.

The method of microimmunoelectrophoresis provided for the setting of two controls: 1) IPS towards allantoic and amnyotic fluid from not contaminated with grippe virus hen embryos and 2) the sera of 6 rabbits towards the antigens of staphylococcus and grippe virus for the detection of possible natural precipitins towards the two antigens in the rabbit serum.

Results and Discussion

By the method of the double diffusion in gel nine precipitation lines were established between the IPS and PHA employed, three of them rather weak. A clear, although not very strongly manifested precipitation line was noted also with the Staphylococcus aureus antigen. The latter was identified with one of the PHA precipitation lines. A very weakly manifested, transparent line was developed with the tuberculin as well as with the typho-paratyphoid vaccine and with the antigen of the grippe virus A-2. Common antigens between PHA and the rest of the agents employed in the experiment, were not established.

The result was convincing in terms of the discovery of a common antigen between Staphylococcus aureus and PHA, but rather doubtful insofar tuberculin, typho-paratyphoid vaccine and antigen of the grippe virus A-2 were concerned. We could not reject definitively the presence of common antigens between PHA and the infectious agents studied, for the possibilities of the method embarked on were rather limited. The qualities of the IPS used, on one hand, and the quantitative relationships between antigen-antibody fractions, on the other (which, in the antigens employed might not be optimally represented) have both an essential bearing. The lack of precipitation between the normal human serum and PHA in gel with positive Ascoli reaction and immunoelectrophoresis, furthermore emphasize the significance of the factors pointed out and the restricted possibilities of the agar-gel precipitation in the investigations herein reported.

The results of the microimmunoelectrophoresis are presented in figures 1 and 1′.

The IPS employed produces six precipitation lines of PHA, two of them being blurred and rather wide and corresponding to the lines, produced by PHA and not-immune rabbit and human sera; the remainder are thin and clearly outlined, within the zone of PHA α and β fractions.

As illustrated by the figures enclosed, the Staphylococcus toxin displays a clear-cut and well-outlined, tiny precipitation line within the zone of the α-globulin fraction of PHA. The grippe antigen exhibits a wide, diffuse precipitation zone which corresponds to the zone of the α-fractions of PHA and a second precipitation line which is fine, clear-cut and well outlined. Both precipitation lines appearing between IPS and the grippe antigen, correspond to the respective precipitation lines between the latter and PHA; the former resembles in nature the precipitation zone produced by the PHA with normal rabbit and human sera. The set controls for IPS

(anti PHA) — allantoic and amnyotic liquid of hen embryos, not contaminated with grippe virus, yielded negative results. Negative is likewise the result of the control setting, undertaken with the aim to establish natural

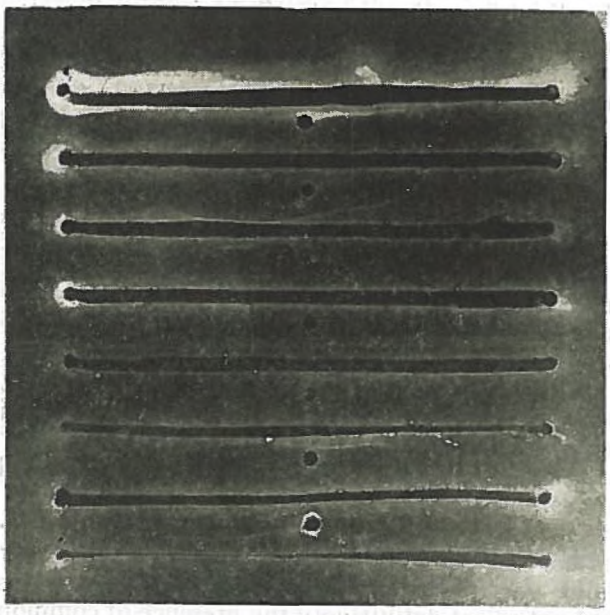

Fig. 1. Immuno-electrophoregram of the PHA, Staphylococcus aureus, Streptococcus ß haemolyticus and grippe virus A-2 antigens, produced by IPS-anti PHA. Micro-immuno-electrophoresis in agar-gel, veronal buffer pH 8.2, 250 V, 150 mA, duration — 6 hours. Staining with amido-schwarz.
1 — PHA;
2 — Staphylococcus aureus antigen;
3 — Streptococcus β haemolyticus antigen;
4 — Grippe virus A-2 antigen.

precipitins to the antigens of the Staphylococcus and grippe virus in the rabbit sera.

Insofar antigenic unity between Streptococcus β haemolyticus and PHA is concerned no evidence whatsoever resulted from the experiment herein described.

The results provide sufficient evidence for the presence of common antigens between the toxin investigated of Staphylococcus aureus and PHA, as well as between the antigen of the grippe virus and the PHA.

In the discussion of the results, worth of special interest appear to be the data of the studies made by Ling and assoc. (19) who established a sub-

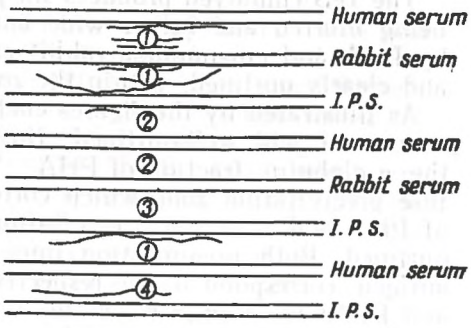

Fig. 2. Scheme of the micro-electrophoregram.

stantial mitogenic activity of a Staphylococcus aureus product outside the cell (possibly toxin), similar to that caused by PHA. Ling and Husband (18) in the course of comparative studies on the mitogenic PHA activity and the antigens of Staphylococcus aureus, tuberculin, tetanic toxin etc., found out a mitogenic effect of the antigens, isolated from the Staphylococcus, very close to that of the PHA. The observations of the authors just cited are an indication, although indirect, for a possible immunological proximity between PHA and Staphylococcus. Our results provide for a definitive explanation of these findings.

Staphylococcus and grippe infections are widely spread among humans and therefore, immunocompetent cells exist towards their antigens, manifested in variable degree. These cells, subjected to the effect of PHA and particularly, to its antigens, related to the staphylococcus and grippe, react by proliferation and blastic transformation.

The following data corroborate the latter conclusion of the authors: the lymphocytes of the rat and guinea pig are not altered under the effect exerted by the Staphylococcus antigen. The PHA, likewise, does not exert a mitogenic effect upon the rat's lymphocytes — they are immuno-non-competent equally towards PHA and towards Staphylococcus antigens (18).

The data obtained by the authors concerning the antigenic unity between PHA from Phaseolus vulgaris, sort Sax, toxin of Staphylococcus aureus and the grippe virus antigens demonstrate that one of the mechanisms of mitogenic activity of PHA constitutes in essence a secondary immunologic response. By accepting such a possibility, we, by no means reject the additional, generally stimulating properties of the PHA. It is very likely that the phenomenon studied be induced by independent and yet, concomitant and exerting mutual influence factors.

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АНТИГЕННАЯ ОБЩНОСТЬ МЕЖДУ ФИТОГЕМАГГЛЮТИНИНОМ ИЗ PHASEOLUS VULGARIS И НЕКОТОРЫМИ БАКТЕРИЯМИ И ВИРУСАМИ

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РЕЗЮМЕ

Установлена антигенная общность фитогемагглютинина (ФГА) из Phaseolus vulgaris со Staphylococcus aureus и гриппозным вирусом A_2. Идентификация проведена при помощи иммунного анти-ФГА кроличьей сыворотки крови, путем методов двойной диффузии в агаре и микроиммуноэлектрофореза. Эти результаты показывают, что сильно выраженная митогенная активность ФГА и ее индивидуальные колебания у отдельных лиц, может представлять собой вторичную иммунологическую реакцию сенсибилизованных к этим широко распространенным инфекционным агентам лимфоцитов. Этим не отвергается возможное общее стимулирующее действие ФГА.