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The Changes of Collagen Synthesis Following the Active Arthus Reaction in the Guinea Pig Skin

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ABSTRACT. Active Arthus reactions were provoked by injecting 100 μg bovine serum albumin (BSA) into the skin of sensitized guinea pigs. Skin biopsy samples were taken 1, 4, 8, 24, 48, 72 and 120 hours after the provocation. Samples were labeled by intradermal injection of 10 μCi [3, 4-^{3}H]- proline for 1 hour and specific activity of radioactive proline per amount of protein was determined on 1 N NaOH extract of dermis as total protein synthesized. And the synthesized collagenous protein was measured as ^{3}H-hydroxyproline by the method of Juva and Prockop. The results were compared with those obtained by the control injections of 100 μg BSA into the skin of non-sensitized guinea pigs.

The synthesized total protein increased at 1, 4 and 8 hours, decreased at 24 hours and increased again at 72 hours. The time course of collagenous protein synthesis was nearly the same as that of total protein synthesis, however the synthesized collagenous protein was higher than the total protein synthesized 1 and 4 hours after the provocation.

It was suggested that collagen synthesis increased in an active Arthus reaction up to 4 hours after the provocation.

Key words : Immune complex — Active Arthus reaction — Fibroblast — Collagen synthesis

Recently, many authors reported that factors derived from lymphoid cells stimulate fibroblast migration and proliferation and collagen and noncollagen protein synthesis.[1-4] However, it has not been sufficiently investigated yet how proliferation or collagen and noncollagen protein synthesis of fibroblast are influenced by immunologically induced inflammation. Fibrotic changes of dermis are one of the histological characteristic of scleroderma and erythema elevatum diutinum, which are suggested to relate to immune response and found circulating immune complexes and various autoimmune antibodies. Immune complexes induce a series of inflammatory reactions by activating complement system. It is very interesting how the reactions influence proliferation and metabolism of fibroblast. In this study, we observed the changes of collagenous and total proteins synthesis following the Arthus reaction in the guinea pig skin.

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MATERIALS AND METHODS

Animals

Male, Hartley strain, albino guinea pigs weighing 400–500 g were used.

Active Arthus Reactions

Guinea pigs were sensitized by injecting 1 mg Bovine serum albumin (BSA ; Sigma, St. Louis, Mo., USA), emulsified in Freund's incomplete adjuvant, into their necks. Three weeks later, a booster injection of 1 mg/ml of HRP solution was administered subcutaneously into the neck of each animal. Seven days after the booster injection, sera were obtained from the sensitized guinea pigs and examined for antibody titer using the double diffusion agar gel method. Guinea pigs which showed the same titer of precipitating antibody were selected for induction of active Arthus reactions. The highest dilution of antigen solution precipitable with antibody was 2^5 times the original solution which contained 1 mg/ml of HRP.

Active Arthus reactions were provoked by injections of 100 μg BSA in 0.1 ml PBS (0.01 M phosphate buffered saline, pH 7.2) into the footpad skin of sensitized guinea pigs.

Controls

Injections of 100 μg BSA in 0.1 ml PBS into the footpad skin non-sensitized guinea pigs were performed as controls.

Biopsies

Skin biopsy samples were taken from both sensitized and non-sensitized guinea pigs 1, 4, 8, 24, 48, 72 and 120 hours after the injections. Skin samples from both sensitized and non-sensitized guinea pigs which did not receive injections were also taken.

Measurement of Synthetic Activity of Collagenous and Total Proteins

Ten μCi L-[3,4-^{3}H]-proline (New England Nuclear, 36.8 Ci/m mole) in 0.1 ml PBS was injected intracutaneously at the provoked sites 1 hour before biopsies. The dermis was immediately taken from the skin samples manually, and then was homogenized. Two ml of 1 N NaOH was added to each homoginate, and the preparations were stirred. One-half ml samples from each preparations were used for assay of protein content. To the remaining sample, TCA was added to a final concentration of 10%, and TCA insoluble precipitates were collected. The precipitates were then hydrolyzed in a seeled Pyrex tube with 2 ml of 6 N HCl at 125–135°C for 6 hours. The hydrolysates were dried in vacuo and dissolved in 4 ml of distilled water. Aliquots of 0.1 ml were transferred into counting vials, mixed with 10 ml Insta-gel (Packard Instr. Co., Illinois, USA), and the radioactivity determined. The radioactivity was considered a measure of the amount of total protein synthesized. Hydroxyproline-associated radioactivity was assayed in the remaining hydrolysate, according to the method of Juva and Prockop[5] and was considered a measure of the amount of collagen synthesized. The percentage of total protein synthesized as collagen was calculated from the radioactivities in total protein synthesized and in collagenous protein synthesized, according to the assumption of Wiestner et al.[6]

Measurement of Protein

Protein concentration was measured according to the method of Lowry et al.[7] using bovine serum albumin as a standard.

All data from each group and observation time were analyzed statistically with Student's t-test.

RESULTS

Radioactivity of [^{3}H] proline incorporated into total and collagenous proteins and the ratio of synthesis of collagen to total protein of each group and observation time are summarized in Table.

Synthesis of total protein increased at 1, 4 and 8 hours after the provocation of active Arthus reaction, and the rates of increase were approximately 46% to 122% of the value of non-provoked skin. At 24 hours after the provocation it decreased by approximately 48% of the value of non-provoked skin, and increased again at 72 and 120 hours. The time course of synthesis of collagenous protein following active Arthus reaction was nearly the same as that of synthesis of total protein. However, the increase at 1 and 4 hours was significant and the rates of increase were approximately 170% and 150% of the value of non-provoked skin, respectively.

On the contrary, when 100 μg BSA was injected into the non-sensitized guinea pig skin, synthesis of total protein slightly increased at 1, 8 and 72 hours, and the rates of increase were approximately 11%, 38% of the value of non-injected skin, respectively, and decreased at 120 hours by 50% of the value of non-injected skin, respectively, and decreased at 120 hours by 50% of the value of non-injected skin. The time course of synthesis of collagenous protein showed nearly the same pattern as that of total protein synthesis.

At 1, 8 and 72 hours after the provocation of active Arthus reaction, the ratio of synthesis of collagen total protein was higher than those obtained by the injection of 100 μg BSA into the non-sensitized guinea pig skin.

DISCUSSION

The data obtained in this in vivo experiments suggest that collagen synthetic activity increases in the early phase of Arthus reaction. Microscopically, obvious edema of the upper dermis and small mononuclear cell infiltration, are seen at 1 hour after the provocation. At 4 hours after the provocation, there are conspicuous extravasations of erythrocytes and severe infiltrations of polymorphonuclear leukocytes.[8]

It is very interesting to know which factors increase collagen synthetic activity in the early phase of an Arthus reaction. Some chemical mediators or infiltrative cells, appearing in the inflammatory reactions at the sites of Arthus reactions probably concern this phenomenon. It has been reported that prostaglandin E_1, E_2[9] and heparin[10] enhance the collagen synthesis, on the other hand, histamin[11] suppresses it. As mentioned, it is suggested that the factors derived from lymphoid cells and macrophages[12] playing the important role in immunologically induced inflammation.

It is indicated that the collagen synthesis decreases transiently 24 hours after the provocation of active Arthus reaction. Recently, the mouse macrophage elastase has been reported to play a role in the physiological remodeling of connective tissues.[13,14] We suppose that there are the similar regulating

TABLE The changes of total protein and collagen synthesis following the active Arthus reaction

	0 hr	1 hr	4 hr	8 hr	24 hr	48 hr	72 hr	120 hr
Incorporation of [^{3}H]-proline into total protein synthesized (10^2 dpm/mg·protein)								
Arthus Group+	677±101(8)	1504± 76(7)**	1492±201(8)*	986± 98(10)	354±37(8)*	604±65 (8)	1138±129(10)	1241±235(8)*
Control Group‡	656±105(8)	730±100(8)	707± 91(8)	908±123(10)	594±48(8)	508±90(10)	739±131 (8)	328± 45(8)
Incorporation of [^{3}H]-proline into collagenous protein synthesized (10^2 dpm/mg·protein)								
Arthus Group+	236± 42(8)	635± 87(8)*	596± 87(8)*	347± 59(10)	126±10(8)*	293±38 (8)	553± 65 (9)*	416± 76(8)**
Control Group‡	240± 44(8)	235± 36(8)	260± 32(8)	248± 27 (9)	206±19(8)	227±26(10)	280± 49 (9)	110± 14(8)
Ratio of synthesis of collagen to total protein (%)								
Arthus Group+	13.9	16.9	16.0	14.1	14.2	19.4	19.4	13.4
Control Group‡	14.6	12.9	14·7	10.9	13.9	17.9	15.2	13.4

+ Active Arthus reactions were provoked by intradermal injections of 100 μg BSA to the footpads of sensitized guinea pigs.

‡ Intradermal injections of 100 μg BSA to the footpads of non-sensitized guinea pigs.

Tests were carried out in 7 to 10 samples. Each value represents mean±S.E. Difference from control : * $P<0.05$ ** <0.01

mechanisms on the tissue at 24 hours after provoked active Arthus reaction.

The re-increase of total and collagenous protein 72 and 120 hours after the reaction might be explained by reparative process of inflammation.

In the future, we hope to study the factors which increase collagen synthetic activity in the early phase of an Arthus reaction.

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