The effect of corticosteroid therapy on lysosomal enzymes and protein and lipid metabolism in rabbit lung after administration of Freund's adjuvant

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Summary

The effect of corticosteroid therapy on the broncho-alveolar cell response, the changes in activity of some lysosomal enzymes and the protein and lipid biosynthesis rates in lung tissue of normal rabbits and of rabbits after induction of an acute inflammation by the intravenous injection of complete Freund's adjuvant (CFA) 0,2 ml/kg body weight was investigated.

Three intramuscular injections of methylprednisolone acetate (Depo-Medrol) 1,2 mg/kg over a period of 8 days reduced the mean total broncho-alveolar free cell yields significantly. The percentages of lymphocytes and granulocytes were decreased. The increase in the macrophage percentage was associated with a significant increase in the acid phosphatase activity of the broncho-alveolar cells. The ß-glucuronidase activity, on the other hand, was lowered in alveolar cells and even significantly suppressed in lung tissue. Protein and lipid biosynthesis was significantly retarded in lung tissue 8 days after the start of therapy.

Administration of a single dose of CFA 0,2 ml/kg evoked an acute lung inflammation and a significant increase in total alveolar free cell yields. The macrophage percentage was reduced and the lymphocyte numbers doubled, whereas the granulocyte percentage increased more than sevenfold. The change in the percentage distribution of granulocytes may be associated with the marked increase in ß-glucuronidase activity of the cells as well as of the lung tissue. In the inflammatory phase, protein biosynthesis was significantly increased but lipid synthesis was not affected.

Corticosteroid therapy in animals treated with adjuvant reversed all the effects of CFA. It has very pronounced anti-inflammatory action and a catabolic effect on protein and lipid metabolism.

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Recently it was shown that intramuscular corticosteroids had a dramatic effect on the cytological and histopathological features associated with acute extrinsic allergic alveolitis induced in rabbit lung by injection and follow-up inhalation of horseradish peroxidase.[1] Immunization of rabbits with complete Freund's adjuvant (CFA) followed by exposure to nebulized horseradish peroxidase also caused an acute hypersensitivity pneumonitis which in some cases resembled the syndrome seen in human patients.[2] CFA injected subcutaneously into rabbits caused only a few foci of congestion in the lungs. However, CFA injected intravenously induced cell mobilization in many lung areas to the point of almost complete consolidation.[3] In many ways this reaction resembles the one evoked by immunological mechanisms and consists of a dense cellular infiltrate of mononuclear cells, polymorphonuclear leucocytes, lymphocytes, a few plasma cells and a proliferation of type II pneumocytes.[4] It is suggested that the peribronchial and interstitial lymphoid tissue which contains B, T and null lymphocytes may trigger the initial response releasing soluble factors that attract mononuclear and polymorphonuclear cells.[5] The release of lysosomal enzymes by these cells onto the bronchiolar and alveolar surfaces is a potential source of inflammation, leading to lung disease.[6]

Apart from the beneficial effect of corticosteroids on allergic alveolitis, they are commonly used to treat a variety of conditions associated with acute lung injury, generally described as the respiratory distress syndrome.[7] In animal experiments corticosteroids have been shown to induce lymphopenia and an almost complete disappearance of the bronchus-associated lymphoid tissue (BA LT), a reduction in the number of lymphocytes in the alveolar lavage and clearance of the intra-alveolar and interstitial reactions.[1] Subcutaneous administration of cortisone acetate (100 mg/kg body weight for 7 consecutive days) induced a decrease in the percentage of T lymphocytes and macrophages in the broncho-alveolar lavage.[8] Corticosteroids also decreased the weight of adult rat lungs with oxygen toxicity[9] and after prolonged administration[10] induced mechanical changes in the pressure-volume characteristics and the structure of the type II cells.

Investigations of the effect of corticosteroids on lung metabolism are few. Hydrocortisone administered twice daily for 7 days had no significant effect on lung weight or DNA and total phosphatidylcholine content.[11] Other researchers[12] found an increase in total phospholipids and phosphatidylcholine content after corticosteroid therapy.

Corticosteroids have a differential effect on protein synthesis in muscle.[13] Slow-twitch red fibres are not affected. In mixed fibre type muscles a 50-60% reduction in the rate of protein synthesis was found to be due to a loss of RNA and inhibition of translation, resulting from an impairment of peptide-chain initiation.[13] The effect on lung protein synthesis is unknown.

In view of the dramatic effects of long-acting corticosteroids on the broncho-alveolar cells and BA LT of rabbits immunized with horseradish peroxidase,[1] the role of corticosteroid therapy was investigated during acute lung inflammation induced in

rabbits by the intravenous injection of CFA. The following parameters were studied: (*i*) the type and yield of free broncho-alveolar cells; (*ii*) acid phosphatase (AP) and ß-glucuronidase activity of the broncho-alveolar cells and lung tissue; and (*iii*) the rate of protein and lipid biosynthesis by lung tissue after treatment of the animals with a corticosteroid, CFA and a combination of the two.

Material and methods

New Zealand White rabbits (1,5-2,0 kg) were used. The animals were accustomed to the animal house for at least 8 days before the experiment was started.

The rabbits were divided at random into four groups of 5 animals each. Group A served as a control. Group B received intramuscular injections of methylprednisolone acetate (MPA) (Depo-Medrol) 1,2 mg/kg on the 1st, 3rd and 6th day. Group C was injected via the ear vein with CFA 0,2 ml/kg on day 1. Group D was treated with both MPA and CFA, the dosage being similar to that used in the animals in groups B and C.

On the morning of the 9th day, 1 animal from each group was anaesthetized by an intravenous injection of sodium thiopentone. The lungs and heart were dissected *en bloc* and transferred to ice-cold saline. Using an endotracheal cannula, 40 ml ice-cold 0,9% saline was infused and aspirated four times and the recovered lavage fluid stored on ice in separate siliconized tubes. This procedure was repeated six times, giving a total volume of 240 ml lavage fluid per lung. The tubes containing the lavage fluid were centrifuged for 15 minutes at 700 *g* and the supernatants were discarded. The pellets of cells were transferred to one tube and dispersed in a total volume of 10 ml saline.

The lungs were stored in ice-cold saline until further processing. Total cell counts were made with a Spencer Brightline haemocytometer. For cytological purposes a few drops of dispersed cells were put on object glasses and allowed to settle for 3 minutes. The excess fluid was removed with filter paper and the air-dried preparations were fixed and stained with Leishman stain. A minimum of 200 cells per preparation were counted. The following cell types were identified in the differential counts: (*i*) macrophages; (*ii*) lymphocytes; (*iii*) neutrophils; (*iv*) eosinophils; and (*v*) basophils. Epithelial and red blood cells were not recorded. To determine viability of the cells, the eosin Y exclusion method was used.

Determinations of lavage cells

The cell suspensions were diluted to a concentration of 4 x 10^6 cells/ml. After addition of sufficient Triton X 100 to give an 0,1% solution, 5 ml samples were sonicated for 4 x 15 seconds, using a cell disruptor (Ultrasonics). The tubes were centrifuged at 10 000 *g* for 10 minutes and the supernatants were used for enzyme and protein determinations.

The activities of ß-glucuronidase and AP enzymes were mea-sured in triplicate, according to the methods of Bergmeyer and Gawehn.[14]

Determinations on lung tissue

Lung tissue was sliced with a McIlwain tissue chopper and divided into 300 mg portions. Each portion was suspended in 5 ml de-ionized water containing 0,1% Triton X 100 and sonicated for 4 x 15 seconds. The sonicated samples were centrifuged at 10 000 *g* for 10 minutes and the supernatants were used for the determination of the ß-glucuronidase and AP activity.[14]

The rates of protein and lipid synthesis were determined by measuring the incorporation of ^{14}C-leucine and ^{14}C-palmitate into the proteins and lipids of lung slices respectively over a period of 2 hours, as described previously.[15]

Protein was determined[16] on: (*i*) protein extracts with 0,8M KCl tris HCl (low-ionic-strength soluble protein); and (*ii*) the cell and tissue supernatants.

DNA[17] was determined on accurately weighed samples of whole lung tissue. Each experiment was repeated five times. All estimations were done in triplicate and standard methods were used to compute the mean and standard error of the mean. Pairwise comparisons were made using a two-sided Student's *t* test.

Results

In Fig. 1 the mean broncho-alveolar free cell yields from the lungs of 2 rabbits sacrificed at different time intervals after intravenous injection of CFA 0,2 mg/kg are recorded. A marked increase in the total free cell yield is seen with time. The dramatic increase is nearly linear up to 11 days, at which time the total number of free cells in the lavage fluid increased more than eightfold above the mean control value.

In Table I the mean total cell yields and the differential counts of the cells in the lavage fluid from control rabbits (group A) and
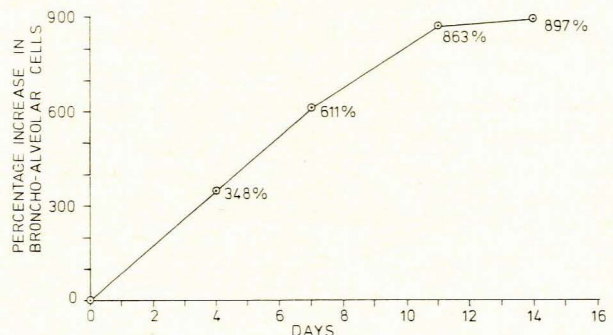

Fig. 1. Percentage increase in broncho-alveolar cells in lavage fluid from rabbit lungs after a single intravenous injection of CFA (0,2 ml/kg).

TABLE I. TOTAL BRONCHO-ALVEOLAR CELL YIELDS AND DIFFERENTIAL CELL COUNTS (MEAN ± SE) IN THE LAVAGE FLUID FROM THE RABBIT LUNGS

Group	Alveolar cell yields (x 10^6)	Differential cell counts (%)		
		Macrophages	Lymphocytes	Granulocytes
A	29,46 ± 5,05	97,52 ± 0,77	1,36 ± 0,62	1,12 ± 0,36
B	10,72 ± 2,27†	98,80 ± 0,71†	0,72 ± 0,30*	0,48 ± 0,18*
C	108,74 ± 15,39†	89,72 ± 1,11†	2,80 ± 0,40*	7,48 ± 1,62†
D	44,32 ± 9,69*	98,40 ± 0,42	0,88 ± 0,52	0,72 ± 0,52

*P < 0,05.
†P < 0,01.

those injected with intramuscular MPA 1,2 mg/kg (group B), CFA (group C), and a combination of MPA and CFA (group D) are recorded. In comparison with the control values, the broncho-alveolar free cell yields were significantly lowered by MPA and significantly increased by CFA. When both MPA and CFA were administered, the increase in cell numbers caused by the action of CFA was counterbalanced by the lowering effect of the MPA. Looking at the percentage distribution of the cells in the differential counts, the numbers of lymphocytes and granulocytes were reduced in the lavage fluid from animals treated with MPA. In group C animals which received CFA there was a significant increase in the lymphocyte and granulocyte percentages. Simultaneous administration of CFA and MPA moderated the elevating and reducing effects of these two substances respectively. The observed changes in lymphocyte and granulocyte percentages were accompanied by a significant increase in macrophages in MPA-treated animals and a significant decrease in animals treated with CFA.

In Table II the AP and ß-glucuronidase activity of broncho-alveolar lavage cells and lung tissue from the different groups of animals are recorded. The results show that MPA elevates AP alveolar cell activity (group B), and this tendency is also exhibited in the alveolar cells of the group D rabbits, which received both corticosteroid and CFA injections. Corticosteroid has a tendency to lower ß-glucuronidase activity in alveolar cells and lung tissue; this latter reduction was significant. In contrast, CFA enhanced the activity of this enzyme significantly in alveolar cells as well as lung tissue.

In Table III the protein content of the alveolar cells and the protein and DNA content of the lung tissue from the different groups are summarized. The protein content of the broncho-alveolar cells and the lung tissue as well as the DNA content of lung tissue from the different groups were not significantly different.

In Table IV the rates of protein and lipid biosynthesis in terms of ^{14}C-leucine and ^{14}C-palmitate incorporation into the lung proteins and lung lipids of the different groups are recorded. Expressed in terms of milligrams of protein or milligrams of DNA as base lines, the rates of protein synthesis were significantly lowered in lung tissue of animals treated with corticosteroid and significantly increased in lung tissue of animals injected with CFA. Compared with controls, the rate of ^{14}C-palmitate incorporation into total lipids of lung tissue was significantly lowered only in the MPA-treated group.

Discussion

In the present investigation an acute inflammatory response was induced in the lungs of rabbits by the intravenous injection of a single dose of CFA. It has been shown that administration of CFA produces a massive interstitial and intra-alveolar infiltrate of cells, proliferation of type II pneumocytes and giant-cell formation as well as cuboidalization of epithelial cells.[18] As far as could be judged from the cell composition of the broncho-alveolar lavage fluids, this response could not be differentiated

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TABLE II. AP AND ß-GLUCURONIDASE ACTIVITY (nmol/mg PROTEIN/MIN) (MEAN ± SE) OR BRONCHO-ALVEOLAR LAVAGE CELLS AND LUNG TISSUE

	AP		ß-glucuronidase	
Group	Broncho-alveolar cells	Lung tissue	Broncho-alveolar cells	Lung tissue
A	38,82 ± 5,72	17,54 ± 1,88	0,85 ± 0,06	0,11 ± 0,01
B	58,54 ± 5,21*	18,30 ± 0,60	0,82 ± 0,13	0,09 ± 0,01*
C	41,42 ± 4,17	21,08 ± 1,55*	1,34 ± 0,15†	0,21 ± 0,02†
D	51,02 ± 10,22†	20,42 ± 1,54	1,12 ± 0,29	0,11 ± 0,01

* $P < 0,05$.
†$P < 0,01$.

TABLE III. PROTEIN AND DNA CONTENT (MEAN ± SE) OF BRONCHO-ALVEOLAR CELLS AND LUNG TISSUE AFTER LAVAGE

	Group			
	A	B	C	D
Protein (mg/10[6] cells)	0,15 ± 0,02	0,14 ± 0,02	0,15 ± 0,01	0,15 ± 0,01
Protein (mg/100 mg tissue)	7,84 ± 0,24	7,80 ± 0,14	7,89 ± 0,20	8,08 ± 0,59
DNA (mg/100 mg tissue)	0,57 ± 0,10	0,60 ± 0,02	0,64 ± 0,08	0,57 ± 0,07

Values in groups B, C and D are not significantly different from those in the control group.

TABLE IV. ^{14}C-LEUCINE AND ^{14}C-PALMITATE INCORPORATION (MEAN ± SE) INTO PROTEINS AND LIPIDS OF LUNG TISSUE

	Group			
	A	B	C	D
[14]C-leucine (dpm/mg protein)	7 373,8 ± 880	5 912 ± 398*	10 484,8 ± 452†	7 917,0 ± 1 019
[14]C-leucine (dpm/mg DNA)	40 620 ± 7 392	31 040 ± 1 753*	54 960 ± 4 993*	38 040 ± 7 014
[14]C-palmitate (dpm/mg DNA)	39 600 ± 3 050	28 800 ± 1 789*	33 000 ± 4 796	34 600 ± 7 829

* $P < 0,05$.
† $P < 0,01$.
Dpm = disintegrations per minute.

from that induced immunologically by intramuscular injection of horseradish peroxidase followed by exposure of the animals to nebulized horseradish peroxidase per trachea.[1] A single intravenous injection of CFA induced a progressive cell mobilization in the rabbits' lungs which reached its maximum about 14 days after injection (Fig. 1). For this reason we measured the broncho-alveolar cell response, the biochemical changes in enzyme activity and the biosynthesis capacity of lung tissue 8 days after injection.

It is generally accepted that corticosteroids have a short-term and a long-term effect. In the present experiment, corticosteroid therapy induced a dramatic reduction in the mean total broncho-alveolar cell numbers of normal animals treated every 3rd day with a dose of MPA 1,2 mg for 8 days. It has a similar effect on broncho-alveolar cells mobilized by immunological mechanisms.[1] It also reversed the elevation of the total alveolar cell numbers induced by a single injection of CFA. The corticosteroid reduction in total broncho-alveolar cell population might be due to its suppression of lymphoid tissue activity and of the mononuclear phagocyte system. It has been reported that corticosteroid therapy leads to a general lymphopenia and also suppresses the BALT of rabbit lung.[1]

CFA also has a marked influence on the differential cell distribution in the alveolar lavages, giving rise to a twofold increase in the percentage of lymphocytes, whereas the granulocytes increase nearly sevenfold compared with control values. In general it would appear that the cell response to CFA is typical of an acute inflammation in the lung, as described earlier.[18]

The results of corticosteroid therapy on the activity of acid hydrolases indicate a significant increase in AP activity in the alveolar cells, but only a slight elevation in that of the lung tissue itself. Its effect on ß-glucuronidase activity was totally different. Instead of producing an elevation corticosteroid reduced the concentration of ß-glucuronidase in lung tissue significantly with a similar trend in alveolar cells. CFA had no clear-cut effect on the AP activity of alveolar cells, but caused it to increase significantly in lung tissue. On the other hand, it induced a significant elevation of ß-glucuronidase activity in both alveolar cells and lung tissue.

In the light of our finding that corticosteroid therapy inhibited protein synthesis or enhanced protein degradation in general, it would be difficult to offer an explanation for the increase in one protein enzyme and the inhibition of the other. However, the observed differences between the activities of AP and ß-glucuronidase after corticosteroid and CFA administration appeared to be linked to: (i) the described changes in percentage distribution of free cells in the lavage fluid; and (ii) differences in the relative concentrations of these two enzymes in macrophages and granulocytes. The increase in AP activity of alveolar cells after corticosteroid therapy could be due to the relative increase in macrophage numbers concomitant with a reduction in granulocyte numbers (Table I). On the other hand CFA, which induced an increase in the granulocyte percentage in the broncho-alveolar lavage fluid, caused a marked elevation in ß-glucuronidase activity; whereas AP activity was not significantly affected. No information is available about the relative concentration of AP and ß-glucuronidase in macrophages and granulocytes respectively. From our results it would appear that there is a high concentration of AP in macrophages, whereas in granulocytes ß-glucuronidase is predominant.

Information regarding the effect of anti-inflammatory steroid drugs on the release of acid hydrolases from macrophages and polymorphonuclear leucocytes is conflicting,[18] but because of their stabilizing effects on lysosomal membranes (especially in short-term therapy) one would expect an increase in lysosomal enzymes, whereas long-term therapy had a catabolic effect and retarded cellular metabolism.[19] In view of the high turnover rate of broncho-alveolar cells, one would expect to find only short-term effects on these cells even after administration of long-acting corticosteroid.

From our results of the effect of corticosteroid therapy on the rate of protein biosynthesis, it is clear that MPA inhibited protein biosynthesis in lung tissue significantly. This finding is corroborated by the effect of corticosteroid on protein synthesis in mixed fibre type muscles[13] where an inhibition of translation was demonstrated, resulting in a 50-60% reduction in the rate of protein synthesis. It is also interesting to note that the total protein and DNA content of alveolar cells and lung tissue were not statistically different in the various groups (Table III). A similar observation on short-term corticosteroid therapy was made previously.[11]

The rate of ^{14}C-palmitate incorporation into lung lipids was also significantly slowed in lung tissue of adult rabbits treated with MPA. CFA had no significant effect. The observed lowering of the rate of lipid synthesis by MPA could be due to its stabilizing effect on the cell membranes. If that is so, the intracellular pool of ^{14}C-palmitate could be reduced to levels which will slow down lipid synthesis.

However, other researchers[11] found no significant effect when hydrocortisone was administered daily for 7 days, whereas yet others[12] claimed an increase in total lung phospholipids and phosphatidylcholine content after corticosteroid therapy. It would therefore appear that the type and dose of corticosteroid and the duration of therapy as well as the animal species employed may determine the final effect on lung metabolism.

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