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THE EFFECT OF CORTICOSTEROIDS ON SUBCUTANEOUS ABSCESS FORMATION IN THE MOUSE

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Summary.—We have used a recently developed model for s.c. abscess formation to study the effect of corticosteroids on abscess formation in mice. Mice were given daily i.p. injections of either hydrocortisone, 20 mg/kg/day or dexamethasone, 0·8 mg/kg/day, starting 3 days before inoculation with *Staph. aureus* and continuing for the duration of the experiment. Another group of mice was given a single injection of dexamethasone, 8 mg/kg, 1 h after inoculation with *Staph. aureus*. Encapsulated abscesses developed in all animals by Day 4, and there was no mortality. Abscess volume ± s.e.mean at 4 days was reduced ($P < 0{\cdot}0005$) from 39·9 ± 3·0 mm³ in controls to 16·7 ± 3·6 mm³ in the daily dexamethasone group. Abscess volume at 4 days after a single dose of dexamethasone was 39·9 ± 8·0 mm³. Bacterial concentrations per ml of pus were equivalent in all groups ($10^{10{\cdot}6}$–$10^{10{\cdot}9}$). The effect of steroids on formation of sterile abscesses was also studied. Abscess volumes were smaller in animals given daily hydrocortisone or dexamethasone when compared to controls, but the difference was significant only for mice receiving daily hydrocortisone. These results suggest that prolonged high-dose steroid administration decreased the magnitude of the acute inflammatory reaction responsible for abscess formation in the soft tissue but did not interfere significantly with the process of containment and encapsulation of s.c. abscesses. A single massive dose of steroid did not influence abscess formation.

CORTICOSTEROIDS IN HIGH DOSAGE appear to have a profound effect on the acute inflammatory response, but the activity of these agents on abscess formation has not been defined. We have developed a quantitative model in mice for encapsulated s.c. abscess formation using an inoculum of either *Staph. aureus*, *B. fragilis* or autoclaved caecal contents (Joiner *et al.*, 1980*a*). Employing this model in mice with selective immunological defects, we showed minimal alteration in abscess formation in animals that were complement-deficient or athymic (Joiner *et al.*, 1980*b*). In the present report, we describe the effect of hydrocortisone succinate and dexamethasone phosphate on s.c. abscess formation in mice challenged with *Staph aureus*.

MATERIALS AND METHODS

Animals.—Male CD-1 outbred mice (Charles River Breeding Park, Wilmington, Mass.) weighing 28–30 g were used for all experiments. Mice were housed in groups of 5–10 per cage and maintained on chow and water *ad libitum*.

Inocula.—The source of *Staph. aureus* was a blood culture from a patient with bacteraemia and was the same strain (VA-1) used in previous experiments. The organism was grown for 18 h at 37° in peptone–yeast–glucose (PYG) broth (Scott Robbins Laboratories, Fiskville, RI), quick-frozen in liquid N_2 and stored at −60°. The fresh broth culture of *Staph. aureus* was adjusted to 3×10^8 cfu/ml.

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Mice received an (s.c. injection of: 1) PYG broth culture of *Staph. aureus* combined with an equal volume of autoclaved mouse caecal contents; or (2) autoclaved mouse caecal contents combined with an equal volume of sterile PYG broth. Both inocula are known to produce abscesses in normal mice. Details of inoculum preparation and implantation have been reported previously (Joiner *et al.*, 1980*a*). Briefly, mouse caecal contents from grain-fed mice were diluted 1:3 in PYG, filtered through coarse surgical gauze, autoclaved at 121° for 2 h, and frozen until used. Inocula of 0·25 ml were injected s.c. with a tuberculin syringe 1 cm lateral to the spine and just anterior to the left hind extremity. All animals were active immediately after this procedure.

Corticosteroid administration.—Dexamethasone sodium phosphate (D) (Merck, Sharp & Dohme, Rahway, NJ) and hydrocortisone sodium succinate (H) (Upjohn, Kalamazoo, MI) were employed. These drugs were diluted in phosphate-buffered saline (PBS) and injected i.p. in a volume of 0·2 ml. Three different regimens were administered to separate groups of mice: (1) hydrocortisone sodium succinate, 20 mg/kg i.p. daily, starting 3 days before challenge, and given for a total of 7 doses (daily H). (2) Dexamethasone sodium phosphate, 0·8 mg/kg i.p. daily starting 3 days before challenge, and given for a total of 7 doses (daily D); and (3) dexamethasone sodium phosphate, 8 mg/kg i.p. in a single dose, given 1 h after challenge (single D). Control mice received 0·2 ml sterile PBS i.p. daily for 7 days.

Evaluation of abscesses.—Abscess size was evaluated on the 4th day after challenge using caliper measurements of external lesions in living animals and volume measurements of abscesses after killing. Caliper measurements were made using the longest diameter and the corresponding perpendicular diameter. The product of these values was used to determine external area. For volume measurements, the animals were killed and the abscesses were removed by wide dissection. Abscesses were fixed in formalin for 48–72 h and the lesion was then sectioned along the midline at the greatest diameter. The diameter of the entire abscess in a plane perpendicular to the initial section was measured with calipers. One of the abscess halves was stained with haematoxylin and eosin and with aniline blue (collagen stain) for histological assessment. The cross-sectional area of the histological specimen was measured by planimetry with a Grafpen sonic digitizer (Design Data, Inc., Cambridge, MA) and a Hewlitt Packard 9830A digital computer. Abscess volume was calculated by the following formula:

$$\text{Abscess volume} = \text{Cross-sectional abscess area on histological section} \times \frac{\text{Perpendicular diameter measured with calipers}}{2}$$

Evaluation of histological sections by light microscopy in previous experiments showed 3 distinct zones. The inner zone, comprising the abscess centre, contained necrotic white blood cells, fibre from the inoculum, and bacteria. Surrounding this central region was a distinct

TABLE—*Abscess dimensions measured by planimetry in corticosteroid-treated mice*

Inoculum	Drug	External area (mm^2) ± s.e.	Abscess area mm^2 ± s.e.	WBC area mm^2 ± s.e.	Capsule area mm^2 ± s.e.	Abscess volume mm^3 ± s.e.
ACC + *Staph. aureus*	Hydrocortisone (daily H) n = 8	61 ± 6	7·4 ± 1·3	3·5 ± 0·7	1·6 ± 0·2	16·7 ± 3·2
		$P < 0·0005$*	$P < 0·0005$	$P < 0·0005$	$P < 0·0005$	$P < 0·0005$
	Dexamethasone (daily D) n = 8	68 ± 7	9·7 ± 1·5	4·3 ± 0·6	2·8 ± 0·5	19·3 ± 3·6
		$P < 0·0005$	$P < 0·0005$	$P < 0·05$	N.S.	$P < 0·0005$
	Dexamethasone (single D) n = 4	97 ± 7	14·1 ± 2·1	7·3 ± 1·2	3·0 ± 0·3	39·9 ± 8·0
		N.S.	N.S.	N.S.	N.S.	N.S.
	Control n = 8	100 ± 6	13·9 ± 0·7	6·9 ± 0·6	3·3 ± 0·5	39·8 ± 3·0
ACC	Hydrocortisone (daily H) n = 6	28 ± 9	4·5 ± 1·4	2·8 ± 0·6	0·6 ± 0·3	8·5 ± 3·9
		$P < 0·005$*	$P < 0·02$	$P < 0·02$	$P < 0·00005$	$P < 0·05$
	Dexamethasone (daily D) n = 6	35·6	6·7 ± 0·8	3·1 ± 0·4	1·6 ± 0·5	13·5 ± 2·7
		$P < 0·005$	$P < 0·05$	$P < 0·02$	$P < 0·05$	N.S.
	Control n = 6	57 ± 2	8·3 ± 0·7	4·3 ± 0·5	2·3 ± 0·1	17·7 ± 1·7

* As compared to the control group.

band of stainable leucocytes, more than 95% of which were polymorphonuclear cells. The third zone was a discrete collagen capsule which encompassed the white cell band and was well formed by the 4th day after challenge. The cross-sectional areas of these 3 zones were measured by planimetry.

Quantitative bacterial counts of abscess pus were performed on abscesses that were aseptically removed by wide dissection. An aliquot of 0·1 ml of pus was added to 9·9 ml of sterile PBS and homogenized by vortexing. Serial 100-fold dilutions were made, and 0·1 ml aliquots of each dilution were plated on trypticase sheep blood agar. Colonies were counted after 24–48 h, and results expressed as cfu/ml pus.

Statistics.—Student's *t*-test was used for calculations of statistical significance.

RESULTS

All mice developed abscesses by the 4th day after inoculation, and no deaths were observed. In untreated animals the abscess was larger in those receiving *Staph. aureus* compared with those given autoclaved caecal contents (ACC).

Abscesses from animals challenged with inocula containing *Staph. aureus* were significantly smaller in mice given daily D or daily H compared to those in untreated animals (Table). A single injection of dexamethasone, however, had no effect on abscess size or volume. The differences observed in daily-D and daily-H groups applied to external area, cross-sectional area, and abscess volume. Sterile abscesses formed with an inoculum containing ACC and sterile broth were also smaller in animals given daily D and daily H compared to the lesions in untreated mice. The daily-H and daily-D groups showed no important differences, except that external area was significantly greater in the infected abscesses in the daily-D group.

On external appearance, abscesses in animals receiving steroids were not as sharply defined as those in untreated groups. This especially applied to mice inoculated with ACC alone which showed small, flat lesions with indistinct borders. Furthermore, abscesses in the steroid-treated animals were soft in consistency, quite in contrast to the firm, sharply delineated nodular lesions which developed in untreated mice.

The leucocyte area generally paralleled abscess area, comprising 44–62% of the total cross-sectional area. Significantly smaller WBC areas were measured in mice given daily H or daily D when compared to controls; this difference was noted in abscesses in animals challenged with either *Staph. aureus* or ACC.

Steroid therapy did not alter the percentage of abscess area occupied by the capsule, with one exception. The mean capsule area was extremely small, $0{\cdot}6 \pm 0{\cdot}3$ mm^2 ($\pm$ 1 s.e.), in mice challenged with ACC and given daily H, representing only 13% of total abscess area. Not only was this the smallest capsule area in any group in this experiment, it was the smallest observed in any of the studies with this model, including those in C5-deficient mice, cobra-venom-factor-treated animals, and congenitally athymic (nude) mice (Joiner *et al.*, 1980*b*).

Quantitative counts of bacteria in abscess pus were equivalent in all groups. The mean CFU/ml $\pm$ s.d. for daily-H, single-D and control mice were $1 \times 10^{10{\cdot}9 \pm 0{\cdot}2}$, $1 \times 10^{10{\cdot}70 \pm 0{\cdot}3}$ and $1 \times 10^{10{\cdot}60 \pm 0{\cdot}3}$ respectively.

Weight loss and splenic atrophy occurred in mice receiving daily steroid injections. The mean weight of mice given daily H or daily D decreased by 8% (2·3 g) and 9% (2·8 g) respectively. The weight of untreated animals fell from 29·7 g to 29·2 g (2%), but the weight of mice receiving a single dose of D did not change. Average spleen weights at the time of killing in the daily-H, daily-D and single-D groups were 0·119 g, 0·092 g and 0·196 g respectively. The mean weight of spleens from untreated animals was 0·199 g, which is similar to the splenic weight of healthy animals of the same age without abscesses.

DISCUSSION

The effect of steroids on cutaneous abscess formation has received only cur-

sory attention. In a retrospective clinical study, staphyloccal infections, particularly those with bacteraemia, appeared to be more frequent and more severe in patients receiving corticosteroids (Cluff *et al.*, 1968); the origin of these infections was frequently a cutaneous site. Agarwal (1967) studied the effects of steroids on a rapidly evolving, dermonecrotic lesion in mice which was produced by s.c. injection of *Staph. aureus* and cotton dust. Mice were given 1–4 doses of either cortisone acetate, prednisolone, dexamethasone, or betamethasone. In all cases, the last injection of steroids preceded inoculation with *Staph. aureus* by at least 30 min. Tissue weight and the leucocyte influx 4 h after inoculation were markedly decreased in all groups, except in animals given cortisone. Larger zones of necrosis were observed at 24 and 48 h after inoculation in the steroid group; this effect was partially dependent on the number of staphylococci injected, since lesions from inocula containing only 1×10^2 to 1×10^3 *Staph. aureus* were not affected by cortisone. Bacterial counts in the lesions of cortisone-treated mice were no different from those in controls at 24 h after inoculation.

Steroids have a profound effect on the inflammatory response associated with abscess formation. These drugs cause a peripheral neutrophilic leucocytosis (Fauci, Dale and Balow, 1976). In high dosage they suppress phagocytosis and bactericidal capacity of neutrophils *in vitro* (Dale and Petersdorf, 1973; Hirsch and Church, 1961; Mandell, Rubin and Hook, 1970), but this effect is not generally observed *in vivo* (Allison and Adcock, 1965; Glasser, Heustis and Jones, 1977). More importantly, steroids suppress leucocyte accumulation at an inflammatory site (Allison, Smith and Wood, 1955; Dale, Fauci and Wolff, 1974; Perper *et al.*, 1974). Using the Rebuck skin window method in humans Boggs *et al.* (1964) demonstrated that both neutrophil and monocyte accumulation were decreased when a single 200mg dose of cortisol phosphate was given 2–8 h before abrading the skin. Compared with the controls, there was no difference in inflammation when steroids were given concurrently with or following abrasion of the skin. Leucocyte accumulation was also suppressed after a 24 h continuous infusion of cortisol, or during a 10-day period of high-dose oral prednisone therapy. The most likely explanation for these effects is that corticosteroids reduce granulocyte adherence to vascular endothelium after an inflammatory stimulus (Ebert and Barclay, 1952; MacGregor, Spagnulo and Lentnek, 1974).

Glucocorticoids decrease collagen synthesis *in vivo* and *in vitro* when used in pharmacological concentrations (Kivirikko *et al.*, 1975; Newman and Cutroneo, 1978; Smith, 1967). A recent study in mice demonstrated a 50–70% reduction in collagen synthesis in sponge granulomas when dexamethasone (0·35 mg/kg/day) was given for 2 weeks (Kruse *et al.*, 1978). Hydrocortisone may have decreased formation of the collagen wall in sterile abscess in this study.

Mice, rats and rabbits are considered corticosteroid-sensitive species, with a marked tendency to lympholysis in the presence of glucorticoids (Claman, 1972). Man is considered to be a corticosteroid-resistant species. Caution must be used when applying the results from this study to humans, since studies which show profound effects on the inflammatory response in a corticosteroid-sensitive animal do not necessarily apply to man.

A single large dose of corticosteroids is frequently used for treating patients with septic shock. This single dose is generally felt to have few adverse effects on host defences against infection, and it may improve survival in bacteraemic shock (Schumer *et al.*, 1976). Our study suggests that s.c. abscess formation at 4 days is unimpaired after a single large dose of dexamethasone. Furthermore, this study suggests that more prolonged (1-week) steroid administration in mice does not interfere significantly with the process

of containment and encapsulation of s.c. abscesses.

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