

Immunofluorescence of the skin in gold rashes – with particular reference to IgE

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SUMMARY Immunofluorescence studies have been carried out on rashes from 36 patients with rheumatoid arthritis receiving gold therapy. 24 of the rashes were clinically attributed to gold and 12 were diagnosed as coincidental rashes. IgE was found in 6 of the gold rashes and in 4 of the coincidental rashes. Immunofluorescence changes of immune complex vasculitis, lichen planus, or pemphigoid were found in 9 gold rashes while 2 coincidental rashes showed vascular fluorescence for immunoglobulins but not for complement. Two definite gold rashes showing no changes on immunofluorescence showed perivascular infiltration with lymphocytes on light microscopy. Thus, while immunofluorescence is only marginally helpful in the diagnoses of gold rashes, evidence of an immunological reaction tends to favour a diagnosis of a gold-induced rash.

Toxic reactions occur in approximately 32% of patients receiving sodium aurothiomalate (myocrisin) for rheumatoid arthritis (Freyburg *et al.*, 1972). Dermatitis accounts for 60% of these reactions (Empire Rheumatism Council Research Subcommittee, 1960). It is often difficult to distinguish drug-induced rashes from coincidental rashes developing during treatment or from rashes unnoticed until cryotherapy had focused attention on the skin.

Davies *et al.* (1973) found raised serum levels of IgE in patients with toxic reactions to gold and suggested that type I allergic mechanisms might play a part in the pathogenesis of gold-induced skin rashes. This raised the possibility that the detection of IgE within the skin itself, or a study of its distribution, might be of value in the diagnosis of such rashes. We present the results of a histological and immunological study of skin lesions from 36 patients with rheumatoid arthritis who developed skin rashes while receiving gold therapy.

Materials and methods

Patients with classical or definite rheumatoid arthritis, who developed or first noticed rashes during gold therapy, were referred to the Department

of Dermatology for clinical diagnosis. A biopsy and blood for serum IgE levels were taken. One-half of the biopsy specimen was sent for immunofluorescence studies and the other half for routine histology.

IMMUNOFLUORESCENCE

Methods used in the preparation of tissue sections from quick frozen, unfixed blocks of skin and the washing and fixation before reaction with fluorescein isothiocyanate (FITC)-labelled antisera have been described previously (Scott and Rowell, 1975). Standard methods for direct and indirect immunofluorescence were used in the immunohistological staining of sections and in tests for specificity of staining (Nairn, 1969). FITC-labelled conjugates were used in concentrations which produced no nonspecific staining in sections of normal human skin— $\frac{1}{8}$ U/ml in the case of the anti-IgG and anti-C3 conjugates and 1 U/ml in the case of the anti-IgA and anti-IgM conjugates. Unitage was assayed by the gel diffusion precipitation test of Beutner *et al.* (1970). Stained sections were examined in incident light with a Leitz Orthoplan microscope using a combination of three BG38 and one GG47 filters in addition to the number 3 turret filters and K530 suppression filter.

The direct staining procedure was used for the detection of IgG, IgM, IgA, and C3 in sections. FITC-labelled anti-IgG (lot no. K.7248), anti-IgM

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(lot no. K.8819), and anti-IgA (lot nos. K.7307 and K.8778) were purchased from Burroughs Wellcome, and FITC-labelled anti-C3 (lot no. F.5175) from Hoechst Pharmaceuticals.

The indirect procedure was used for the detection of IgE. Unlabelled rabbit anti-IgE (lot no. 2049H) and FITC antirabbit globulin (lot no. K5863) were obtained from Hoechst Pharmaceuticals and Burroughs Wellcome respectively. Before its use in staining experiments the anti-IgE serum, which produced strong agglutination of human erythrocytes, was absorbed twice with one-quarter of its own volume of human group AB erythrocytes. The serum was used at a dilution of 1:15 and the FITC antirabbit globulin at a dilution of 1:60. Controls used in indirect staining experiments consisted of sections pretreated with unlabelled normal rabbit serum (1:15 before their exposure to FITC antirabbit globulin).

ESTIMATION OF SERUM PROTEINS

Hyland Immunoplates were used for the estimation of serum levels of IgG, IgM, IgA, and C3 by radial immunodiffusion. The radio-labelled radial immunodiffusion technique of Rowe (1969) was used for the estimation of serum levels of IgE.

Results

During the study 36 patients with rheumatoid arthritis developed or first noticed rashes while receiving gold therapy. 19 of these had clinically classified rashes (see below) attributable to gold (group A). In a further 5 patients (group B) the rashes were too faded for clinical characterization but all 5 had recently developed signs or symptoms strongly suggestive of a gold rash and the drug had been withdrawn. The remaining 12 patients were considered clinically to have coincidental rashes not attributable to gold (group C). Permission to biopsy unaffected skin was obtained from 4 patients in group A.

GROUPS A AND B

There were 16 women and 8 men, with a mean age of 50.7 years. Sodium aurothiomalate dosage varied from 20 mg to 3.6 g (mean 859 mg). The rash had been present before biopsy from 10 days to 28 weeks (mean 6.8 weeks). Five types of rash were seen (Table) resembling (a) pityriasis rosea 4, (b) eczema, usually discoid 9, (c) lichen planus 2, (d) psoriasis 1, (e) exfoliative dermatitis 1. The rashes were mixed in 2 patients (pityriasis/discoid eczema; pityriasis/lichenoid). In 5 stomatitis accompanied the rash. 5 rashes were too faded for accurate diagnosis (group B).

Table Morphological classification of rashes seen in 36 patients with rheumatoid arthritis on gold therapy

	Groups A and B: gold rash (n = 24)	Group C: coincidental rash (n = 12)
Eczema	9	6
Pityriasis	4	0
(pityriasis + lichenoid)	1	
(pityriasis + eczema)	1	
Lichenoid	2	0
Psoriasiform	1	1
Exfoliative dermatitis	1	0
Too faded for diagnosis	5	0
Stomatitis + rash	5	0
Urticaria	0	2
Ichthyosis	0	1
Seborrhoeic dermatitis	0	2

GROUP C - COINCIDENTAL RASHES

There were 7 women and 5 men, with a mean age of 51.3 years. The dose of gold administered had varied from 200 mg to 3.7 g (mean 1.24) and the rash had been present from 3 to 100 weeks (mean 25 weeks). Eczematous rashes were seen in 6 (discoid 2, varicose 1, nonspecific 3), urticaria in 2, ichthyosis 1, psoriasis 1, seborrhoeic dermatitis 2. Seven of these rashes were studied histologically. 5 showed a nonspecific dermatitis, 1 lichen sclerosis et atrophicus, 1 neurodermatitis.

LIGHT MICROSCOPY

Sixteen rashes considered definitely to be due to gold were studied by light microscopy. Chronic or nonspecific dermatitis was reported in 14, but 2 had changes suggestive of lichen planus, in neither of which was the rash clinically thought to be lichenoid. Changes were generally unremarkable. Slight eosinophilic infiltration was seen in one biopsy which also showed lichenoid changes. Perivascular infiltration by lymphocytes was a common feature of the nonspecific dermatitis but it was only marked in 3 biopsies from the gold rash group.

IMMUNOFLUORESCENCE

IgE was detected no more frequently in rashes from group A and B patients than in rashes from group C patients. It was found in 5 skin lesions from group A patients, in 1 of 5 lesions from group B patients, and in 4 of 12 lesions from group C patients.

Biopsies of clinically affected and unaffected skin were obtained from 4 group A patients. In 3 no IgE was detected in either the affected or unaffected skin. In 1 fluorescence for IgE was detected in both biopsies. Fluorescence for IgE was not usually strong and no characteristic distribution of IgE serving to differentiate group A and B patients from group C patients emerged.

IgE was found in the endothelial lining or in the walls of capillaries and on connective tissue fibres in

the papillary and upper reticular layers of the dermis. It was found also in the cytoplasm of an occasional mononuclear cell lying either close to blood vessels or free in the connective tissue spaces. In one instance the cells had the appearance of mature plasma cells but in others they remained unidentified. The presence of IgE was not associated with any particular type of skin rash nor was it associated with histological evidence of an eosinophilic or macrophagic infiltrate.

Granular deposits of IgG, IgM, and C3 were found in and around the walls of blood vessels in lesions from 5 group A patients and 2 group B patients. In the lesions from 1 group A and 1 group B patient extracellular fluorescence for IgE was seen. Deposits of immunoglobulins but not of complement were found in the walls of blood vessels in biopsies from 2 group C patients. Deposits of IgG, IgM, and C3 were also found in the walls of blood vessels in one of the four biopsies of nonlesion skin from group A patients.

Aggregates of IgM, IgA, and C3 lying below, at, and above the dermoepidermal junction and presenting appearances similar to those of lichen planus were seen in 2 of the group A rashes showing an immune complex vasculitis, and in one additional group A rash.

Tubular pemphigoid-like basement membrane zone fluorescence for IgG and C3 was seen in affected but not in unaffected skin from one group A patient. A heavy infiltrate of unstained mononuclear cells was seen around the walls of blood vessels but no immunoglobulins were detected in the vessel walls in lesions from 2 group A patients. The cells were identified as lymphocytes on light microscopy, and the lesions were attributed to type IV allergic mechanisms.

SERUM IGE LEVELS

These were measured in 30 of the 36 cases at the time of the skin biopsy—the interval between this and the last injection of gold varying from 1 to 12 weeks (mean 3.5 weeks). IgE level was <125 IU/ml in 25. Of the remaining 5, the highest level detected was only 600 IU/ml.

EOSINOPHIL COUNT

Differential white cell counts were performed in 20 cases, and found to be abnormal in only 3—one from each main group. Both tissue and blood eosinophilia (18%) occurred in 1 case and eosinophilias of 17% and 12% in the 2.

NONGOLD RASHES

Skin biopsies from 5 rashes developing in patients without rheumatoid arthritis and attributed to drugs

other than gold were examined. One patient had received chlorpropamide, 1 allopurinol, 1 clindamycin, and 2 patients had been on several drugs. There was no immunohistological abnormality in the mixed drug eruptions, but light microscopy of one showed features of a fixed drug eruption. The allopurinol rash in a patient with leukaemia was associated with deposits of IgG and IgA at the dermoepidermal junction. The clindamycin rash showed fluorescence for C3 in capillaries, and the chlorpropamide rash showed the appearances of lichen planus.

Discussion

We attempted to determine whether immunofluorescent tests for IgE might have some diagnostic value in the investigation of skin rashes thought to be attributable to gold therapy in rheumatoid arthritis. They appear to have no such value. IgE was found only in 25% of rashes clinically attributed to gold therapy. The presence of IgE was not associated either with any particular type of clinical presentation or with any specific changes on light microscopy. 19 of the patients studied had active rashes clinically attributed to gold therapy, but only one. Persistently raised serum IgE levels therefore do not appear to be a concomitant of gold-induced rashes in rheumatoid arthritis.

Our findings do not deny the possibility that type I allergic mechanisms may initiate gold-induced skin rashes, as suggested by Davies *et al.* (1973), but they implicate a mechanism other than type I allergy in the maintenance and progression of the skin lesions.

Doubt has been expressed that gold reactions have an immunological basis. Many rashes appear to be dose-related (Walzer *et al.*, 1970) and do not recur on restarting gold therapy (Penneys *et al.*, 1974). Similarly, Almeyda and Levantine (1971) found no evidence that drug-induced (including gold) lichenoid eruptions have an immunological basis. Nonetheless, drug allergy in general (Assem and Vickers, 1972; Müller *et al.*, 1973) and gold rashes in particular (Davies *et al.*, 1973) tend to be associated with raised serum IgE levels. Raised serum IgE, however, has been found in rheumatoid arthritis itself (Hunder and Gleich, 1974) and in other conditions not usually attributed to type I allergic mechanisms, namely pemphigoid and leprosy (Arbesman *et al.*, 1974). Cell-mediated immunity has been implicated in the pathogenesis of gold-induced rashes by Denman and Denman (1968) but the evidence is conflicting (Davies *et al.*, 1973).

Our studies do not clarify the role of immunological mechanisms in the pathogenesis of gold

rashes in rheumatoid arthritis. No evidence of an immunological reaction was found in rashes from 12 of the 24 group A and B patients studied, and these rashes included 3 of the most florid and obvious clinical examples of gold toxicity. However, they do tend to implicate immunological mechanisms in the pathogenesis of at least some of the gold rashes studied. Pemphigoid-like basement zone fluorescence for IgG and C3 was found in the rash from one group A patient and vascular deposits of immunoglobulins and C3 were found in the rashes from 5 group A and 2 group B patients. In 2 of the group A rashes showing an immune complex vasculitis and in 1 additional group A rash the changes of lichen planus were present. A heavy perivascular lymphocytic infiltrate attributed to type IV mechanisms was present as the sole change in rashes from 2 group A patients.

Our findings carry some aetiological and diagnostic implications. On the one hand they incriminate immunological mechanisms in the pathogenesis of only a proportion of gold rashes in rheumatoid arthritis. On the other hand, they indicate that evidence of an immune complex vasculitis or of conditions involving type II mechanisms e.g. pemphigoid, may (a) help to differentiate between gold-induced and coincidental rashes in rheumatoid arthritis, and (b) contraindicate restarting gold therapy.

The failure to detect immune complexes in our group C patients conflicts with the findings of Larsson and Lithner (1972) and with those of Conn *et al.* (1976) and Schroeter *et al.* (1976), who found immune complexes in dermal blood vessel walls in the majority of patients with rheumatoid arthritis. This probably reflects differences in the site chosen for biopsy. Vascular deposits of immune complexes are found with the greatest frequency in biopsies of skin from the lower leg (Schroeter *et al.*, 1976). Our biopsy specimens were taken from various sites depending on the lesion, but seldom from the lower leg. Similarly in the studies of Muijs Van de Moer and Cats (1976), who also failed to detect immune complexes in dermal blood vessels in rheumatoid arthritis, biopsies were taken from the forearm.

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