In Vitro Interferon-Gamma Release Test in the Diagnosis of Drug-Induced Erythema Nodosum

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Erythema nodosum is a skin reaction manifested by tender or painful erythematous subcutaneous nodules, located usually on the extensor aspects of the lower extremities. Histologically, a mononuclear inflammatory cell infiltrate occurs in the septa of the subcutaneous fat lobules. It is likely that erythema nodosum results from an immunologic reaction that can be triggered by a wide range of antigenic stimuli, including drugs, benign and malignant systemic diseases, and bacterial viral and fungal infections [1]. Frequently, the cause is unknown.

Although drugs have been implicated as the cause of erythema nodosum, the etiologic relationship between the drug and the skin disorder is often unclear [2]. In view of recent studies implying that drug-related T cell activity may be assessed by *in vitro* cytokine release tests [3,4], the role of drugs in inducing erythema nodosum was evaluated in the present study by interferon-gamma release from peripheral blood lymphocytes.

Patient Description

A 45 year old woman presented with the sudden appearance of painful subcutaneous nodules, several centimeters in diameter, on the extensor aspects of both legs. Faint erythema was noted on the overlying skin. The patient did not have fever or arthralgia and the rest of the physical examination was unremarkable. The clinical picture was consistent with the diagnosis of erythema nodosum. Histologic examination of skin lesions was not performed. The patient's past history revealed hypothyroidism and infertility. Laboratory examinations were normal or negative, including complete blood count, blood chemistry, chest X-ray, antinuclear factor, rheumatoid factor, complement levels C3 and C4, immunoglobulin E level, and abdominal and gynecologic ultrasound.

The list of medications taken by the patient 1 month prior to the apearance of erythema nodosum included estradiol valerate tablets (Progyluton®) taken for 2 weeks; intramuscular progesterone injection (Gestone®) for 1 week and progesterone suppositories for 1 week. One day before the appearance of of the nodules, the estradiol valerate tablets were reinstituted. At the same time, the patient reported having taken paracetamol tablets (Dexamol®).

The clinical findings, drug history, the temporal relationship to drug intake and the absence of other known etiologic factors for the disorder suggested the diagnosis of drug-induced erythema nodosum. Remission of the skin lesions was noticed 4 weeks after she stopped taking estradiol valerate and began systemic treatment with corticosteroids and diclofenac sodium. In an attempt to identify the incriminating drug(s) responsible for the induction of erythema nodosum, *in vitro* drug sensitivity was studied, based on the release of IFNγ from the patient's peripheral blood lymphocytes following *in vitro* challenge with drugs.

In vitro IFNγ release test

The technique of *in vitro* IFNγ release test developed by us was described previously [3,4]. Briefly, the patient's lymphocytes are cultured for 24 hours in medium containing PHA-P, 5% fetal calf serum with or without the tested drug (unmodified, dissolved in the appropriate solvents). The three maximal drug concentrations that are non-toxic for the lymphocytes are used. Following incubation, the supernatants are collected for the detection of IFNγ release, using a commercial enzyme-linked immunosorbent assay kit (Quantikine kit, R&D Systems, USA). For each drug the increase in IFNγ release (in percentage) is calculated.

The IFNγ release test was used to investigate the drugs that the patient had taken previously: estradiol valerate, progesterone and paracetamol. Individuals exposed to these drugs without known adverse drug reactions served as matched control patients. A threshold level was defined as the mean percentage increase of IFNγ release measured in control patients + 2SD. A maximal value of IFNγ release higher than the threshold level was determined as a positive IFNγ test result [3,4]. In addition to matched control patients, a threshold level was calculated also for a pool of 40 drugs, excluding paracetamol and non-steroidal anti-inflammatory drugs, taken by 19 control patients (12 males and 7 females).

The maximal IFNγ release values for estradiol valerate, progesterone and paracetamol recorded in the patient were compared to the specific threshold levels for these drugs recorded in the various control patients. Based on this comparison a positive IFNγ release test result was determined for estradiol valerate, whereas negative test results were determined for

IFNγ = interferon-gamma

Table. *In vitro* IFNγ release for drugs in a patient with erythema nodosum and in control patients

Drug	Clinical suspicion	*In vitro* IFNγ release (%IFNγ increase) Patient Maximal	Control patients Mean ± SD	Threshold level (mean + 2SD)	Test result
Estradiol valerate Concentrations (μg/ml): 0.1, 0.5 and 1 Dilutions: 1:2 and 1:5	High	103	6.4 ± 16.6*	39.7	Positive
Progesterone Concentrations (μg/ml): 0.1, 0.5, 1 and 5 Dilutions: 1:2 and 1:5	Low	-21.2	11.2 ± 8.6**	28.4	Negative
Paracetamol Concentrations (μg/ml): 50 and 100 Dilution: 1:2	Possible	61.5	16.7 ±30.1***	76.9	Negative
Pool of drugs Excluding paracetamol and NSAIDs	–	–	-5.4 ±24.6****	43.8	–

* One female patient who took estradiol valerate.
** One female patient who took progesterone.
*** Five patients (2 males, 3 females) who took paracetamol.
**** 19 patients (12 males, 7 females) who took 40 drugs, excluding paracetamol and NSAIDs.
SD = standard deviation, NSAIDs = non-steroidal anti-inflammatory drugs.

progesterone and paracetamol. The threshold level recorded for a pool of 40 drugs further supports this result [Table].

Comment

Drugs that may cause erythema nodosum are: antimicrobial agents (amoxycillin, penicillin, sulfonamides), bromides, idoines, gold salts, analgesics and antipyretics (including paracetamol), oral contraceptives (estrogens/progesterones) and estrogens [1,2]. An increased estrogen level seems to increase the tendency for erythema nodosum to develop, irrespective of whether the increased level of estrogen is due to pregnancy or to oral contraceptives [2]. Erythema nodosum disappears within a couple of weeks after withdrawal of the causative drug [2].

The possibility of drug-induced erythema nodosum was suggested in the present case by the drug history and the absence of other known causes. The temporal relationship of erythema nodosum to estradiol valerate treatment suggested that the condition was caused by estradiol, although the etiologic role of progesterone and paracetamol could not be excluded. In addition, remission of the skin lesions 4 weeks after the patient ceased taking estradiol valerate supported the causative role of estradiol [2]. Drug sensitivity was further evaluated by the *in vitro* IFNγ release test, which was reported recently as a diagnostic tool in several cutaneous adverse drug reactions induced by a variety of drugs, including hormones [3,4]. The *in vitro* IFNγ release test was positive for estradiol but not for progesterone or paracetamol. Estradiol-induced IFNγ release implies estradiol-specific cell-mediated immunity and may confirm the role of estradiol as the offending drug in erythema nodosum. Furthermore, drug-induced IFNγ release may support an immune pathogenesis of erythema nodosum with a polarized Th1 immune response, already reported in skin lesions of this disorder [5]. In conclusion, the *in vitro* drug-induced IFNγ release test may serve as a diagnostic tool in drug-induced erythema nodosum.

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How far you go in life depends on your being tender with the young, compassionate with the aged, sympathetic with the striving, and tolerant of the weak and strong. Because someday in your life you will have been all of these.

George Washington Carver (1864-1943), U.S. agriculturalist born into a black slave family.