Immunosuppressive Serum Factors in Patients with Dermatophytoses

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الخلاصة

العوامل المصلية في مرضى الفطار الجلدي يمكن ان تكون أحدى مسببات تثبيط المناعة الخلوية ضد مستضدات الفطر و خصوصا في الحالات المزمنة لعدوى الفطار الجلدي. في حالات معينة مثل عدوى الفطار الجلدي يمكن ان يكون المرض سببا في توليد عوامل مصلية قادرة على تثبيط المناعة الخلوية مما يجعل المرضى عرضة للأصابات المزمنة.

تم تحضير عالق نقي من الخلايا الليمفاوية المعزولة من الدم الممزوج مع مادة مانعة للتخثر والمأخوذة من 15 مريض بعدوى الفطار الجلدي و15 من الأشخاص الأصحاء ظاهريا, لتصبح جاهزة للاستخدام في اختبار التحفز الانشطاري لخلايا أحادية النواة خارج الجسم الحي بواسطة صبغة التترازوليم (MTT) وقد تم عزل هذه الخلايا بطريقة تثفل الكثافة التدريجي.

عدم وجود فروقات معنوية ($P>0.05$) في النتائج المحصلة من حضن مصل مرضى الفطار الجلدي مع خلايا الدم اللمفاوية العائدة لنفس المرضى أو حين تم حضن مصل المرضى مع خلايا الدم اللمفاوية العائدة للأشخاص الأصحاء ظاهريا.

من النتائج التي تم الحصول عليها, وجد أن مصل مرضى الفطار الجلدي خالي من العوامل المصلية المثبطة للمناعة الخلوية ذات التأثير النوعي وغير النوعي وأنه في حالة وجود مثل هذه المثبطات والمسمات بالعوامل المثبطة لخلايا الدم اللمفاوية والناتجة من الفطر الجلدي نفسه, تكون ذات تأثيرموضعي بدلا من التأثير المجموعي. لقد وجد أن مصل هؤلاء المرضى كان ذو تأثير محفز بدلا من التأثير المثبط على خلايا الدم اللمفاوية.

ABSTRACT

Serum factors may be involved in suppression of cell-mediated immunity to fungal antigens in chronic dermatophytoses. In certain cases like dermatophytoses, the infectious process may generate a serum factor that is capable of inducing immunosuppression, thereby the patient susceptible to chronic infection.

Lymphocytes isolated from heparinized whole blood of fifteen patients and fifteen control subjects were used in order to prepare a pure population, ready for use in microculture tetrazolium (MTT) assay to measure the proliferative activity of PBL in patients and control subjects. Lymphocytes were isolated by density gradient sedimentation. Non-significant differences ($P>0.05$) revealed when the patients sera incubated with its own lymphocytes and with controls lymphocytes.

The study found that absence of the serum inhibitory factors with specific and/or non-specific action in patients sera may be associated with the local rather than the systemic action of dermatophyte-derived lymphocyte inhibitory factors. The study also found that patients sera were with stimulatory rather than inhibitory action.

INTRODUCTION :

The hypothesis of the immunosuppressive factors have been described in the sera of patients suffering from a variety of diseases, like uremia, solid tumors, Hodgkin's disease, active tuberculosis, cirrhosis, and multiple sclerosis[1]. Suppression of lymphocyte blastogenic responses has been related to potentially immunosuppressive serum factors in acute Trichophyton mentagrophytes infections produced experimentally in animals.

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It has also been suggested that the lack of inflammation in Trichophyton rubrum infections may be due to dermatophyte – derived lymphocyte inhibitory factors acting locally rather than systemically; also, local antagonism between immediate and delayed hypersensitivity is another possible mechanism of immunosuppression in dermatophytoses[2].
Persistence of infection is frequently accompanied by poor in vitro cell-mediated responses and absent delayed-type hypersensitivity (DTH). It is not clear whether chronic dermatophytoses is due directly to T-cell hyporeactivity or whether it follows modulation of the immune response by suppressor cells or humoral factors such as antigen or antibody. The presence of humoral "blocking factors" is responsible for persistence and, ultimately, for the chronicity of the disease. Humoral factors may interfere with cell-mediated immunity during a chronic dermatophyte infection. The serum factors likely to be involved in such effects are: specific dermatophyte antigen, antibody, or antigen-antibody complexes. Furthermore, high levels of circulating dermatophyte antigen were demonstrated during acute and chronic infections. It is possible that, during chronic infection, the constant release of dermatophyte antigen into circulation may result in the blocking of specific cell-mediated immunity. The above results suggest that competition between cell-mediated immunity and humoral factors, in particular specific antigens, may account for the loss of cellular immunity to fungal infection[3].
The study aims to investigate that in certain cases like dermatophytoses the infection process may generate serum factor that is capable to induce immunosuppression, thereby the patients susceptible to chronic infection.

MATERIALS & METHODS :

Subjects: Fifteen patients infected with dermatophytes were chosen from the Dermatology Outpatient Clinic of Al-Kadymia Teaching Hospital in Baghdad. Clinical and Mycological laboratory diagnosis were made for each patient. Mycological laboratory results, i.e. direct exam and culture, revealed the infection of those patients with different types of dermatophytes. Blood samples of those fifteen patients and fifteen apparently healthy control subjects were collected.
Isolation of lymphocytes: 2ml of anticoagulated blood (heparinized blood was diluted in equal volume of Hanks balanced salt solution (HBSS) "Flow lab., U.K.", and over layered on 4ml of Isopaque-Ficoll (lymphocyte separation medium) "Pharmacia"[4,5].

Microculture tetrazolium assay (MTT) :

2ml of PBL suspension in RPMI medium without FCS was prepared with a concentration of 1-2x10^6 /ml. 45µl of PBL suspension-RPMI medium without FCS was added to each well of 96 well flat-bottom microtiter plate "Sterilin". The first column was left empty which will be occupied by 50µl of D.W as blank. The additions were as following:
-Duplicate wells to each serum inhibitory factors test as following:
i. 45µl of PBL suspension-RPMI medium with 5µl patient heat-inactivated serum by 56C for 30min.
ii. 45µl of PBL suspension-RPMI medium with 5µl control heat-inactivated by 56C for 30min
-Duplicate wells for control negative:
45µl PBL suspension-RPMI medium with 5µl FCS (10% final concentration).
The microtiter plate was incubated at 37Cº in a humidified 5% CO2 atmosphere and complete sterile circumstances in an incubator "Haeraeus". The procedure was completed according to[6].

In order to measure the lymphocytes proliferative percentage, we used the following formula[7] :
Proliferative % = [absorbency of experimental well/ absorbency of control well] – 1 * 100

RESULTS :

Two types of sera ; patients sera (autologous sera) and normal human sera from control subjects were used (Table 1 & Table 2).

Table 1 . MTT readings (O.D) of dermatophytic patients included in this study for: control negative (C-ve), autologous serum of patient (AS), and normal human serum of control (HU).

Sample No.	C-ve	AS	HU
1	0.99	1.71	1.47
2	0.97	1.65	1.24
3	1.21	1.45	1.6
4	1.11	1.23	1.65
5	0.96	1.65	0.87
6	1.4	1.62	1.04
7	0.97	0.98	0.99
8	0.84	1.8	1.68
9	0.88	1.6	1.87
10	1.135	1.78	1.683
11	0.954	1.66	1.291
12	0.836	1.52	1.74
13	0.815	1.76	0.649
14	1.029	0.89	1.64
15	1.141	1.66	1.85

Table 2 . MTT readings (O.D) of control subjects included in this study for: control negative (C-ve), autologous serum of patient (AS), and normal human serum of control (HU).

Sample No.	C-ve	AS	HU
1	0.922	1.501	1.515
2	0.821	0.839	1.35
3	1.021	1.537	1.124
4	1.1	1.454	1.475
5	1.02	1.56	1.96
6	0.94	1.97	1.54
7	0.96	1.87	1.87
8	0.87	0.978	1.01
9	1.21	1.68	0.965
10	1.11	1.98	0.97
11	0.94	1.47	1.69
12	1.2	1.66	1.45
13	0.89	0.98	1.878
14	0.972	1.73	1.43
15	1.02	1.58	1.65

First, the specificity or non-specificity of the serum inhibitory factors that may be present in the sera of the patients was examined.
By applying pooled t-test between autologous serum (AS) incubated with patient's lymphocytes and AS incubated with control lymphocytes, this test indicated the non-significant differences (P>0.05, P=0.3), and in turn the non-specificity.
The absence of specific inhibitory factors let to test the presence of non-specific inhibitory factors in patients sera, by applying pooled t-test between patient's serum incubated with control lymphocytes, and control serum incubated with its own lymphocytes. The test revealed non-significant differences (P>0.05, P=0.31) between them. This indicated the absence of the non-specific inhibitory factors. If they were present in patient's serum, they most inhibit control lymphocytes proliferation in comparison with normal proliferation of these cells when incubated with normal control serum.
To examine the presence or the absence of serum inhibitory factors, each patient's serum was incubated with its own lymphocytes. It may give inhibitory effect on its own lymphocytes, in comparison with normal proliferation of these lymphocytes (patient's lymphocytes) when incubated with normal control serum with no inhibitory factors. The result obtained by pooled t-test, revealed non-significant differences (P>0.05, P=0.1) between them. The patient's serum, instead of the inhibitory effect, it gave higher stimulator effect on its lymphocytes in comparison to control serum over patient's lymphocytes. This indicated the absence of the serum inhibitory factors (Figure 1).

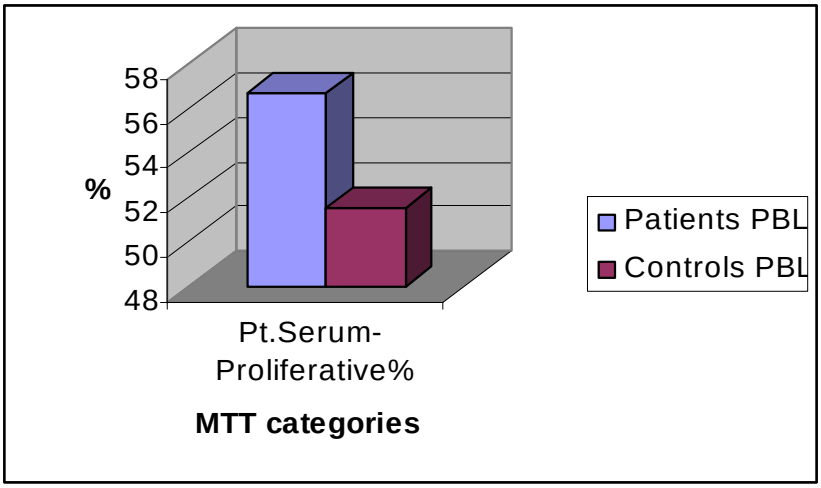

FIGURE 1 . THE PROLIFERATIVE % OF PATIENTS SERA WHEN INCUBATED WITH PATIENTS LYMPHOCYTES AND WITH CONTROLS LYMPHOCYTES.

DISCUSSION :

The hypothesis of the presence of inhibitory factors in the sera of dermatophytic patients might be able to interfere with the cell-mediated immunity developed by the host against the invading microorganism. This has been supported by many previous studies on the suppression of the in vitro lymphocyte transformation due to the presence of such inhibitory factors with specific or non-specific action against patient's lymphocytes. Studies done by Walters et al., in 1974 and Green et al., in 1979[8,9], showed the presence of serum factors which specifically blocked lymphocyte reactivity in vitro in patients chronically infected with dermatophytoses. And that both antigen-specific and non-specific suppression of lymphocyte transformation was related to inhibitory factors in the patient's autologous serum during reinfection.

Such inhibitory factors with specific and/or non-specific action in patients sera enrolled in this study, were absent. This was indicated when the patients sera incubated with control lymphocytes (non-specific action) and patient's serum with its own lymphocytes (specific action).

The absence of the inhibitory factors in our patient's sera was supported by others observation. McGregor et al., in 1992[10], suggested that the lack of inflammation in T.rubrum infections may be due to dermatophyte-derived lymphocyte inhibitory factors acting locally rather than systemically.

The absence of the serum inhibitory factors was also demonstrated by the high proliferation of patient's lymphocytes induced by its own patient's serum, when compared with lower proliferation percentage of these patient's lymphocytes when incubated with normal control serum. This stimulatory rather than inhibitory action of patients' sera could be explained by the presence of growth factors produced by sensitized immune cells in patients sera during the infections. This was also noticed by Dahl, in 1993[11], who found that lymphocytes or monocytes involved in the immune response may produce cytokine growth factors that foster stratum corneum turnover and shedding of the fungus from the skin surface. It may be also explained by the presence of antigen-antibody complexes that instead of inhibiting the CMI, they can act as stimulator for the CMI. As noticed by Vossen et al., in 1995[12] and Marsh et al., in 1997[13], who found that antigen-antibody complexes can crosslink Fc receptors (FcRs) and result in signal transduction events that stimulate and promote the secretion of many cytokines and chemokines.

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