T cell response to anti-CD3 antibody in Down's syndrome

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SUMMARY The non-specific mitogen phytohaemagglutinin (PHA) and an anti-CD3 (OKT3) monoclonal antibody were used to measure the lymphocyte proliferative response in blood samples from 15 subjects with Down's syndrome. Blood from 15 healthy controls closely matched for age and sex was also assayed. The mean blastogenic value in PHA stimulated patient lymphocyte cultures was similar to that calculated in the controls. In contrast, the mitogenic response of lymphocytes from patients with Down's syndrome to anti-CD3 stimulation was on average significantly reduced. Immunofluorescence studies and additional experiments carried out by using semiallogeneic (maternal) monocytes as a source of antigen presenting cells showed that the impaired anti-CD3 induced mitogenesis in Down's syndrome could not be ascribed either to a lack of binding of the antibody to the trisomic cells, or to a defective monocyte-T cell interaction. These findings help to explain the cellular basis of the immune defect in Down's syndrome.

It has been suggested that an intrinsic T cell immune defect in Down's syndrome (trisomy 21) is responsible for the increased susceptibility to infections, malignant diseases, and autoimmune phenomena.¹ Studies of the Down's syndrome lymphocyte proliferative responses to specific and non-specific mitogenic agents, however, have produced conflicting data showing both normal and depressed responses. Data concerning the numbers of circulating trisomic lymphocytes and their immunoregulatory T cell subsets have been more controversial, with results varying from laboratory to laboratory.²

In the early 1980s the mitogenic effect of mouse monoclonal antibodies directed against one surface differentiation antigen—the CD3 antigen complex—was described³ and the crucial role of CD3 antigen in T lymphocyte activation reported.^{4,5} The present investigation was designed to measure the mitogenic action of an anti-CD3 monoclonal antibody on lymphocytes from patients with Down's syndrome. The response to anti-CD3 antibody was then compared with the reactivity of the trisomic cells against the polyclonal T cell activator phytohaemagglutinin (PHA).

Patients and methods

Fifteen patients with Down's syndrome (trisomy 21)

(nine boys and six girls) who were living at home, and 15 karyotypically normal age and sex matched healthy controls were studied. The age range in both groups was 1–30 years; seven subjects were less than 5 years old, four between 6 and 10, and four between 11 and 30. All were free of infection at the time of the study, and no patient or control subject was taking drugs known to affect the immune system.

Peripheral blood mononuclear cells were isolated by Ficoll-Hypaque (Lymphoprep, Nyegaard, Oslo, Norway) density gradient centrifugation, washed three times in Hanks's balanced salt solution and adjusted to a final concentration of 1×10^6 cells/ml in complete culture medium—that is, RPMI 1640 supplemented with antibiotics (penicillin 100 U/ml; streptomycin 100 µg/ml, L-glutamine (2 mM) and 10% heat inactivated fetal calf serum (all from Flow Laboratories, Irvine, Scotland).

Peripheral blood mononuclear cells (1×10^5) were cultured in plastic, 96 well round bottomed culture plates (Nunc, Denmark) at 37°C in a humidified atmosphere containing 5% carbon dioxide in air. PHA-M (Gibco, Grand Island, New York, USA) and an anti-CD3 monoclonal antibody (IgG2a) (OKT3, Ortho, Raritan, New Jersey, USA) directed against a membrane antigen present on most human peripheral T cells as well as on a small

proportion of thymocytes⁶ were used as mitogens. The optimal blastogenic doses of both these reagents were established in preliminary experiments and found to be 1% for PHA and 25 ng/ml for anti-CD3 antibody. Cultures without PHA or anti-CD3 were used as controls. All cultures were performed in triplicate, and the cells pulsed with 0.5 μ Ci (³H) thymidine (specific activity 25 Ci/mmol: The Radiochemical Centre, Amersham, England) after three days of incubation. Twelve hours later incorporation into DNA was measured using a liquid scintillation spectrometer.

An indirect immunofluorescence staining method incorporating fluorescein conjugated goat anti-mouse IgG (Meloy, Springfield, Virginia, USA) as a second labelling antibody⁷ was used to calculate the proportions of CD3 antigen carrying cells in both Down's syndrome and control peripheral blood mononuclear cell preparations.

In the reconstitution experiments, Down's syndrome T lymphocytes (E rosette forming cells⁸) (7×10^5 /ml) were cultured in quadruplicate with plastic adherent semiallogeneic (maternal) monocytes (3×10^5 /ml) in complete medium alone or combined with anti-CD3 antibodies. The incorporation of tritiated thymidine in reciprocal experimental conditions was also assessed. To exclude the proliferation of residual heterologous T cells the cocultured monocyte suspensions were pre-incubated in medium containing 25 μ g/ml mitomycin C (Kyowa Hakko, Kogyo, Tokyo, Japan) for 30 minutes at 37°C. Phorbol myristate acetate (PMA) (Sigma, St Louis, Missouri, USA) was also used at a concentration of 2, 5, and 10 ng/ml.

Statistical analysis was by Student's two tailed *t* test and probabilities of less than 0.05 were accepted as significant.

Results

Although peripheral blood mononuclear cells from three post pubertal subjects with Down's syndrome responded poorly to PHA, the overall mean blastogenic response in our experimental group with Down's syndrome did not differ significantly from that calculated in the PHA stimulated control lymphocyte cultures. In contrast, there was a significant difference between the decreased proliferation induced by anti-CD3 in Down's syndrome and the normal mitogenic value (table 1). It can be seen, however, that a more pronounced defect in cultures with added tritiated thymidine after anti-CD3 stimulus was detected in two patients with Down's syndrome who responded poorly to PHA.

In four experiments a number of cells were available in which to analyse the kinetics of blas-

Table 1 *Lymphocyte proliferative responses to phytohaemagglutinin (PHA) and anti-CD3 monoclonal antibody in patients with Down's syndrome*

Case No	Sex	Age (years)	Net counts/minute (cpm)*	
			PHA	Anti-CD3
1	F	1	83 917†	22 101
2	M	1	86 440	29 848
3	M	3	114 913	18 013
4	M	3	96 913	33 415
5	M	3	100 021	44 984
6	M	4	73 696	16 929
7	M	4	124 419	39 821
8	F	7	119 887	31 717
9	F	8	77 633	19 411
10	M	8	94 824	16 584
11	M	10	119 800	24 624
12	F	16	63 494	10 424
13	F	18	51 601	23 983
14	F	23	66 487	13 817
15	M	30	101 720	38 746
Mean (SEM)—patients:			91 717 (5793)	25 627 (2676)‡
Mean (SEM)—controls:			95 634 (4147)	37 063 (2244)

*Cpm in mitogen stimulated lymphocyte cultures minus background cpm (cpm in unstimulated cultures); †mean of triplicate cultures; ‡p<0.002 (Student's *t* test for unpaired data).

togenesis induced by anti-CD3. The kinetic pattern (seven days of incubation) in Down's syndrome was similar to that of the control lymphocyte cultures, both the cell populations showing a peak response on day 3. Then thymidine incorporation values gradually decreased to reach the starting cpm on day 6 (unpublished data). These findings suggest that anti-CD3 hyporesponsiveness in trisomy 21 is not attributable to the culture conditions.

Immunofluorescence studies were carried out to determine the proportions of CD3 bearing cells in both Down's syndrome and control preparations of peripheral blood mononuclear cells. Phenotypic analysis did not show numerical abnormalities in Down's syndrome: the mean (SD) percentage of anti-CD3 reactive trisomic lymphocytes was 66.5 (5.8), similar to the mean number of control fluorescent cells of 65.4 (5.6).

Mitogenesis induced by anti-CD3 depends on monocytes acting as auxiliary cells.⁹ Reconstitution experiments with heterologous semiallogeneic monocytes were therefore performed to assess the functional integrity of the Down's syndrome macrophage/T cell association. Table 2 shows the results of a representative experiment. Irrespective of the origin of the monocytes, T cells responded to anti-CD3 stimulation according to the reactivity pattern of the unfractionated cells. This observation has been confirmed in two additional experiments.

Table 2 Role of monocytes on anti-CD3 monoclonal antibody induced mitogenesis in Down's syndrome (DS)

Donor cells	Counts/minute* Mean† (range)
Unfractionated (DS)	14 580 (13 020–15 317)
T cells (DS)+monocytes (DS)	12 407 (10 917–13 618)
T cells (DS)+monocytes (mother)	15 938 (13 827–18 614)
Unfractionated (mother)	39 309 (36 777–41 720)
T cells (mother)+monocytes (mother)	33 519 (29 628–36 883)
T cells (mother)+monocytes (DS)	40 444 (39 814–44 624)

*Results corrected for incorporation of thymidine in the absence of monoclonal antibodies to minimise allogeneic helper effects; †quaduplicate cultures.

Discussion

Good experimental evidence suggests that the Down's syndrome lymphocyte proliferative response to PHA mitogen stimulation, which is normal during the first 10 years of life, declines rapidly and irreversibly thereafter.^{10 11} In the present investigation Down's syndrome and control peripheral blood mononuclear cells displayed comparable levels of reactivity to PHA. Values for individual subjects with Down's syndrome varied widely, however, and in three older patients were below the normal range. In contrast, a definite defect in trisomy 21 was seen when the effects of anti-CD3 antibody on the proliferation of resting peripheral blood lymphocytes were assayed. This defect, more pronounced in two postpubertal subjects, was also clearly evident in four children under the age of 10, suggesting that anti-CD3 hyporesponsiveness in Down's syndrome does not depend on age.

The mechanisms concerned in depressed mitogenesis are at present not clear. The lower reactivity to anti-CD3 could not be explained by the absence or paucity of CD3 antigens on the membrane surface of the trisomic cells as the proportion of CD3 bearing lymphocytes in Down's syndrome was similar to that calculated for the control group. T cell subset distribution was not investigated in our trisomic subjects. Conflicting results were reported in Down's syndrome when conventional monoclonal antibodies were used to discriminate helper/inducer (anti-CD4⁺) from suppressor/cytotoxic (anti-CD8⁺) T lymphocytes.^{2 12–15} In addition, these results were often complicated by the fact that the sum of the percentages of cells stained for anti-CD4 and anti-CD8 antibodies exceeded the relative number of anti-CD3 staining cells.^{14 15} A more complete panel of monoclonal reagents together with two-colour immunofluorescence techniques is needed so that the phenotype of mononuclear cell sub-

populations with different functional activities can be identified microscopically. By means of these sensitive methods, overexpanded populations of suppressor (anti-CD8⁺, anti-CD11⁺)² and natural killer (anti-CD8⁺, Leu7⁺)¹⁵ cells have recently been found in the blood of trisomic patients. Both these cells have granular lymphocyte morphology,^{1 16} and presumably perform similar immunoregulatory cell functions.^{16 17} Thus the defective proliferation induced by anti-CD3 and seen by us in trisomy 21 may in part be due to the increased number of cells which inhibit the immune response. Inhibitory mechanisms may also explain other Down's syndrome immunological abnormalities such as the depressed serum titres of 'natural' antibodies^{18 19} and the impaired in vitro specific IgG production in response to recall antigens.²

By culturing lymphocytes from subjects with Down's syndrome with their own mother monocytes, and vice versa, we have shown that an intrinsic T cell immune defect—rather than a deficient helper macrophage function—affected anti-CD3 hyporesponsiveness in patients with Down's syndrome. This was further supported by the detection of an unchanged or decreased proliferative response induced by anti-CD3 in Down's syndrome lymphocyte cultures supplemented with PMA (unpublished data), a substance known to bypass the mechanisms by which accessory cells contribute to T cell growth initiated by anti-CD3 antibody.²⁰

Blastogenesis induced by anti-CD3 is crucially dependent on the production and processing of the lymphokine interleukin 2.²¹ In our laboratories we are evaluating interleukin 2 production and interleukin 2 receptor expression in anti-CD3 pulsed Down's syndrome lymphocyte cultures. Whatever the underlying cellular or molecular defects, or both, our results will provide further evidence that T cell derangement is an integral feature of Down's syndrome.

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