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An intrathyroidal T-cell clone specifically cytotoxic for human thyroid cells

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SUMMARY

We have isolated the first human T-cell clone (IM8) that is specifically cytotoxic to autologous human thyroid cells. In order to obtain primary immunologically reactive cells in patients with autoimmune thyroiditis, we cloned, by limiting dilution, *in vivo* activated intrathyroidal T lymphocytes against thyroid antigens. Clone IM8 expresses CD8 surface antigen, has no activity against autologous or allogeneic mitogen-stimulated lymphoblast cells or allogeneic thyroid cells, and does not lyse K562 cells, implying a lack of natural killer (NK) activity. This observation suggests a role for IM8-like clones in the immunopathology of human autoimmune thyroid disease.

The hallmarks of autoimmune thyroiditis in animals and man are thyroid lymphocytic infiltration and follicle destruction (DeGroot, 1984). It is unlikely that such destruction is caused primarily by a thyroid abnormality since it is possible to induce the disease directly with T-cell transfers between syngeneic susceptible animals (Vladutiu & Rose, 1975; Maron *et al.*, 1983). This, together with the observations of a significant association between thyroiditis and certain MHC antigens, is indicative of an immunological basis for autoimmune thyroid disease (Farid & Bear, 1983). Several recent reports have described T-cell lines and clones (Canonica *et al.*, 1985; Del Prete *et al.*, 1986) prepared from intrathyroidal lymphocytes of patients with autoimmune thyroiditis. In these studies the lymphocytes were selected non-specifically in the absence of thyroid antigens. Upon activation and expansion, these cells were found to be predominantly CD8+ cytotoxic T cells capable of lectin-dependent cytotoxicity or having NK activity. Instead, we selected *in vivo* activated intrathyroidal T cells (ie. blast cells) that were present at the time of surgery by using 40% Percoll density gradients. These blasts were either loosely bound and easily removed by teasing, or more closely associated with the thyroid cells, requiring collagenase digestion to dissociate them (Davies *et al.*, 1985) prior to separation These activated cells were cultured in RPMI and 15% pooled human plasma and 20 U/ml IL-2 (a mixture of 10% Biotest Lymphocult T and 1% Biotest Highly Purified) for 2–7 days before cloning. After cloning by limiting dilution in the presence of IL-2 with autologous irradiated (8000 rads) PMC (10,000/Terasaki well/20 μ l), human thyroglobulin (hTg) and human microsomal antigen (M-Ag) (20 μg/ml) clones were tested for cytotoxicity to autologous peripheral blood PHA blasts, allogeneic blasts, autologous thyroid epithelial cells (TEC), allogeneic TEC, and the K562 cell line. In this way we found the majority of CD8+ clones to lyse HLA-DR mismatched PHA blasts (Mackenzie *et al.*, 1987). We now report detailed studies of one clone (designated IM8) the major specificity of which is cytotoxic activity directed towards autologous thyroid epithelial cells.

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Thyroid tissue was obtained from a patient with goitrous Hashimoto's disease undergoing thyroidectomy for suspected malignancy, and T-cell clones were derived as previously reported (Mackenzie *et al.*, 1987). Phenotypically, clone IM8 is CD8+. When tested against autologous or allogeneic PHA blasts, no lysis was observed (Table 1). Neither was lysis seen against thyroid epithelial cells from Patient 2 (TEC 2) with common HLA A30, B7 and DR9, nor against TEC 3 with common DR2. No NK activity was present as evidenced by lack of lysis with the K562 cell line. Hence, IM8 responds to an antigen that is only expressed in autologous TEC, in contrast to other illustrative clones from the same individual (IM1 and IM2).

When helper and suppressor functions were investigated, it was found that IM8 was suppressive to immunoglobulin (Ig) production, either by direct action on the B cell, or by acting on the T helper/inducer cells as opposed to clone IM20, for example, which exhibited helper function in the presence of thyroid antigen (Table 2). The suppressor activity of IM8 was seen with both autologous (up to 75% suppression) and allogeneic (up to 60% suppression) PMC. Therefore, suppression was not HLA-restricted, and the thyroid specificity of clone IM8 is only apparent in its cytotoxic function.

Although there was no autocytolytic activity, T-cell clone IM8 was weakly autoreactive in terms of a proliferative response to autologous PHA blasts (stimulation index of 6·0)

Table 1. Cytotoxicity studies

	Targets						
	Auto blasts	Allo blasts 1	Allo blasts 2		Allo TEC 2	Allo TEC 3	
Clone	A3, 30 B7, Bw22 DR2, 9	A2, Aw33 B14, B35 DR1, 2	A28, 30 B7, B13 DR6, 9	Auto TEC	A28, 30 B7, B13 DR6, 9	DR2, 6	K562
IM1	1·4±0·5	60·0±5·0	0	0	0	0	0
IM2	52·0±3·0	61·0±4·0	0	24·0±1·2	0	ND	0
IM8	1·2±0·5	0	0	39·0±5·0	5·3±0·2	0	0

T-cell clones were assayed on Day 7 of the feeding cycle, at which time no autologous feeders remained. Cytotoxicity was tested as previously described (Mackenzie *et al.*, 1987). In brief, target cells were labelled with ^{51}Cr for 1 hr at 37° in PBS/5% FBS with shaking at 15-min intervals. 10^5 clone cells were added to 10^4 ^{51}Cr-labelled targets in V-bottomed wells, centrifuged for 2 min at 400 *g* and incubated at 37° for 4 hr. The plates were then centrifuged for 5 min at 400 *g*, and 100 μl of supernatant were removed and counted. Results were calculated as the percentage ^{51}Cr release according to the following calculation:

$$\%\,^{51}Cr\ \text{release} = \frac{\text{specific release} - \text{spontaneous release}}{\text{maximum release} - \text{spontaneous release}} \times 100$$

where spontaneous = targets plus PBS/5% FBS, and maximum = targets plus 20% Triton X100. Data are expressed as mean $\%\,^{51}Cr$ release ± SEM and are typical of two separate experiments. Before testing, TEC, TEC2 and TEC3 were treated for 5 days with bTSH (10 mU/ml, Thytropar 1 U/mg, a gift from USV Pharmaceuticals, Fort Washington, PA) to induce hTg secretion and M-Ag expression, and recombinant human gamma-interferon (100 U/ml) (a gift from Genentech Inc., San Francisco, CA) to induce HLA-DR antigen expression.

Table 2. Helper and suppressor function of T-cell clone IM8

	IgG response (ng/ml)			
	PWM		hTg+M−Ag	
Clone	Auto PMC	Allo PMC	Auto PMC	Allo PMC
IM8	370±122	651±48	202±121	103±24
IM20	1962±7	ND	1343±120	ND
PMC only	1530±48	1106±28	450±10	129±17

T-cell clone IM8 was tested for helper and suppressor function in both non-specific PWM driven system and an antigen-specific system using hTg and M-Ag. T-cell clones were tested on Day 7 of the feeding cycle. 10^4 cloned T cells were added to 5×10^4 PMC, with PWM (0·5%) or a combination of hTg (10 μg/ml) and M-Ag (20 μg/ml). Each test was performed in triplicate in U-bottomed wells in RPMI/15% FBS and incubated at 37° for 6 days with PWM, or for 12 days with thyroid antigen. Supernatant was removed and tested for IgG content by specific ELISA as previously described (Roman, Kom & Davies, 1984).

(Fig. 1) with an apparent decrease in reactivity in the presence of hTg or M-Ag. Hence, the MHC antigens are sufficient to stimulate IM8 to proliferate, but are insufficient stimulus to cause functional cytolytic activity. It is also possible that normal PHA blast cells can present thyroid antigen (such as hTg) present in normal serum. There were insufficient numbers of autologous thyroid epithelial cells available to allow us to look at the proliferative responsiveness of the T cells to autologous thyroid cell surface antigens.

The observation of a thyroid-specific cytotoxic T cell raises the possibility that the initial destructive event in human autoimmune thyroiditis may be thyroid-specific cytotoxicity. We have speculated that a viral infection of the thyrocyte may so change the cell surface antigen that it becomes susceptible to immune damage (Davies, 1985a). Once the initial immune response has begun, the gathering lymphocytic infiltration and resulting gamma-interferon secretion will induce expression of MHC class I and II antigens on the thyroid follicular cell surfaces (Davies, 1985b; Todd *et al.*, 1985; Piccinini, Schachter & Davies, 1986; Piccinini *et al.*, 1987a, b), which will in turn activate further T cells, both MHC-restricted T cytolytic and T-helper cells. In previous studies of intrathyroidal clones from

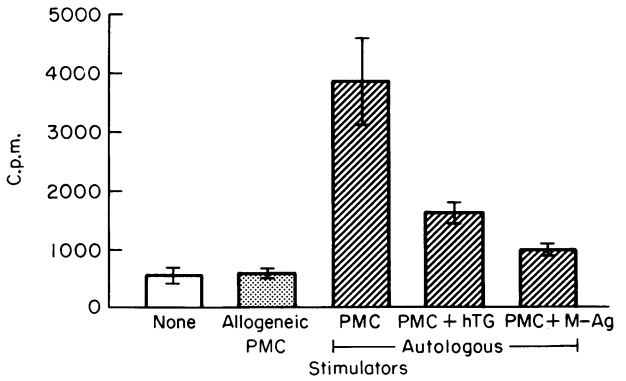

Figure 1. Reactivity of T-cell clone IM8. IM8 cells were tested for their proliferative reactivity to a variety of target antigens as illustrated. T-cell clones were tested at Day 7 of their feeding cycle. 5×10^3 cloned T cells were added to 5×10^4 irradiated PMC, with or without hTg (20 μg/ml) or M-Ag (20 μg/ml). All tests were carried out in triplicate in U-bottomed wells in 15% pooled human plasma/RPMI/penicillin (50 U/ml)/Streptomycin (50 μg/ml). Cells were incubated at 37° for 48 hr, followed by a further 18-hr incubation after the addition of 1 μCi [^{3}H]thymidine. Cells were harvested using a PHD automatic harvester (Cambridge Technology Inc., Cambridge, MA). Data are expressed as the mean c.p.m. ± SEM of triplicate counts.

patients with autoimmune thyroiditis, the majority of T cells were non-specific (Mackenzie *et al.*, 1987), and this would be in keeping with our hypothesis of a thyroid-specific initiation followed by non-specific amplification. Clone IM8 is the first description of a thyroid-specific cytotoxic T cell in human autoimmune (Hashimoto's) thyroiditis, and gives us a better understanding of the mechanisms involved in the complex aetiology of this and other human autoimmune diseases.

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