ALLOREACTIVE MONOFUNCTIONAL HUMAN T CELL CLONES EXPRESSING CYTOTOXIC OR PRIMED LYMPHOCYTE TYPING REACTIVITY AS WELL AS RESTRICTED IMMUNOGENETIC SPECIFICITIES*

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ABSTRACT. Colonies of cloned T cells have been obtained from in vitro primed peripheral blood lymphocytes (PBL) and expanded in order to test their function as well as their immunogenetic specificity. Monofunctional clones have been obtained expressing either cytotoxic (CML) or primed lymphocyte (PLT) reactivity ; each monofunctional colony of cloned T cells expresses a restricted pattern of immunogenetic reactivity as compare to the pattern observed at the population level. Some monoclonal xeno-antibodies raised against an alloreactive T cell line derived from the same alloactivated combination of responder-stimulator, could discriminate between CML clones and PLT clones, while others react with both types of clones but not with the EBV B cell line derived from the same responder or with unrelated B cell lines.

1. INTRODUCTION

Recently introduced progresses in T cell culture allow the continuous growth as a population of human alloreactive T cells using as growth promoters either T cell growth factors (TCGF) (1) or a combination of lectins and irra-

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diated PBL (2, 3). We report here our experience in deriving monofunctional primary colonies expressing immunogenetically restricted reactivities. The obtention of such monofunctional colonies were first searched in order to screen monoclonal antibodies raised against long term cultured alloreactive T cell lines ; the goal using such a sieve was to select those monoclonal antibodies recognizing either cytotoxic or PLT clones as well as those recognizing both, but not the EBV transformed B cell lines derived from the same responder. We report here our preliminary data as well as the use of such T cell clones in the immunogenetic analysis of the human MHC.

2. MATERIAL AND METHODS.

Details have been published and are to be found in the following references. (2, 3 and manuscript submitted).

3. RESULTS

3.1. Screening of the primary T cell colonies for CML or PLT reactivity.

Day 10 to 14 alloprimed cultures of responder A to irradiated stimulator cells B (B^*) express at least 2 different specific immune reactivities, i.e. PLT reactivity (accelerated proliferation towards any cells sharing with the specific stimulator cell HLA-D/DR region determinant(s)) and CML reactivity against any cell sharing with the specific stimulator MHC coded determinant(s). Day 14 AB^* primed cells were restimulated by the cells of the specific stimulator for 48 hours. From such a secondary culture, cloning by limiting dilution was performed in order that the wells of 4 microtiter plates received 4 ± 1.25 primed cells (cloning C1) or 0.25 primed cells per well (cloning C2). Growth was promoted by lectin and irradiated PBL until the first transfer to Cluster plates (Costar, ref. 3524, Costar, Cambridge, Mass. USA), 10 to 14 days after the initial plating. After the transfer to the Cluster

plates of the primary colonies, checked under an inversed microscope, growth promotion was ensured by alternating 2 to 3 cycles using TCGF and 1 or 2 cycles using a lectin plus irradiated PBL. When enough cells could be collected for screening (usually on day 25 to 35), aliquots from each growing colony were tested on both the autologous and the specific cell for CML as well as PLT reactivity. In a typical experiment (cloning C1), 65 growing wells were recorded and transfered to Cluster plates ; 58 grew and, after screening, among these 58 primary colonies, 44 colonies were recorded as PLT positive, CML negative, 1 was found CML positive and PLT negative and 13 were neither cytotoxic nor PLT positive. So far, we have never found primary colonies expressing more than one of the two reactivities we routinely screen for, and none of the reactive colonies were autoreactive.

3.2. T cell nature of the growing colonies : Markers.

92 to 85% of the cells from a given primary colony are E rosette positive ; Ig positive cells (including false positive due to Fc receptors) are less than 1% and 90 to 100% type for the same DR specificities as those expressed on the B cells enriched PBL or the EBV transformed B cell line obtained from the same responder ; this is observed whether the colony is PLT or CML reactive, or not.

Using monoclonal antibodies raised against an alloreactive T cell line, autologous to the cloned combination, we have obtained :

a) monoclonal antibodies specific of all T cells independently of their functions (anti-T cell antibodies) (Table 1, line 4 and 5).

b) monoclonal antibodies specific only of the cytotoxic clones (Table 1, line 1 and 2).

c) monoclonal antibodies specific only of the PLT clones (Table 1, line 3).

Table 1. Different types of monoclonal anti-human T cell antibodies.

	Cytotoxic clone (AB*)	PLT clone (AB*)	EBV derived cell line from A
1 B 32	1925	500	190
2 B 36	2130	480	200
3 B 49	585	2800	175
4 B 51	3658	4429	200
5 B 52	2231	2310	180
6 anti-GAT	498	698	350
7 W6/32 (anti-HLA)	3900	4600	6200
8 6' F10 (species (specific)	1800	1500	560

- Results expressed as mean cpm of triplicates (SD < 5% of the mean).
- Positive control : W6/32 used at dil. 1/100 (a kind gift from Dr. W. Bodmer).
- Negative control = monoclonal anti-GAT (kindly provided by M.Pierres).
- Total cpm introduced per well = 10,000 cpm of a ^{125}I-radiolabelled rabbit anti mouse immunoglobulin.

3.3. T cell nature of the growing colonies : immune reactivity and immunogenetic specificity.

Starting from a population of in vitro primed cells many more colonies with PLT than CML reactivity are obtained. When comparing 4 primary PLT reactive colonies to the original specificity pattern of the primed population (Table 2), we observed that : (a) All colonies have a more restricted response pattern than the original primed population ; (b) some colonies have a response pattern compatible with a classical monospecific D/DR PLT ; (c) Others are reacting with some but not all the cells representing a given D/DR specificity ; (d) Yet others, do not react with the cells of the specific stimulator but with all cells sharing a specific D/DR allele not known to be present on the sensitizing cell. Within families, such clone was nevertheless found to segregate with one haplotype.

Table 2. The PLT reactivity of the original population and of 4 different primary colonies are compared.

Cells of the panel	D/DR	Population (27 anti 4*)	C1.26	C1.9	C1.40	C1.30
1	1/7	35.0	8.4	1.8	3.5	0.1
2	1/7	34.1	8.8	3.8	1.6	0.1
3	1/-	30.9	0.6	2.5	0.08	0.1
4**	1/7	29.7	10.6	6.2	3.5	0.3
5	7/-	28.6	3.8	0.9	2.4	0.1
6	5/4	25.6	12.8	4.9	7.7	1.1
7	1/6	23.9	1.2	2.9	0.1	0.5
8+	a/b 7/-	21.3	6.4	1.9	2.3	0.07
9	5/5	20.3	9.7	4.6	0.6	0.3
10+	c/d 1/2	15.1	8.2	1.0	0.3	1.7
11	1/6	14.4	1.1	3.8	0.8	0.9
12	4/6	14.2	0.3	1.5	0.1	0.03
13++	b/c 1/1	13.9	0.1	0.2	0.05	0.17
14++	a/b 6/1	13.8	1.0	1.7	0.8	0.8
15	2/7	12.7	3.2	0.2	1.0	1.8
16++	c/d 1/2	11.0	1.2	NT	1.1	4.4
17	5/7	10.8	5.0	1.6	0.07	0.3
18+	a/b 1/-	10.1	1.0	NT	NT	1.0
19++	b/d 1/2	10.0	.5	1.3	0.2	3.9
20	1/5	8.5	8.1	0.2	0.9	0.3
21++	b/d 1/2	5.76	0.2	1.6	0.1	2.5
22++	b/d 1/2	5.71	0.1	0.3	0.02	1.4
23++	a/c 6/1	3.1	0.2	0.3	0.02	0.1
24++	a/c 6/1	2.5	0.3	0.2	0.6	0.05
25++	a/d 1/2	2.2	0.5	0.5	0.3	2.4
26++	a/d 1/2	1.7	0.1	0.7	0.07	4.0
27*	6/8	0.4	0.4	1.1	0.03	0.09

Details of the PLT technique are fully described in Ref. 3 and in (1). Results are expressed as mediane of cpm x 10^{-3} (pulse of ^{3}H TdR between day 3 and day 4).

* Positive control = responder cell

** Negative control = specific stimulator

+ or ++ refer to individuals from 2 families with corresponding haplotypes a, b, c and d.

(1) B. MALISSEN, D. CHARMOT and C. MAWAS. 1980. Expansion of human lymphocyte populations expressing specific immune reactivities. III. Specific clones, either cytotoxic or proliferative, obtained from a population of responder cells primed in vitro. Immunogenetic analysis. Submitted.

In parallel, the screening for the specificity of one cytotoxic primary colony showed that this colony was directed toward HLA-B-7 and its cross-reactive group (in this experiment, HLA-B 22 - Table 3).

Table 3. Fine specificity of the cytotoxic clone C2-4 using a cold target inhibition assay (Responder = F ; Stimulator = C*).

Cold targets used	HLA type			Number of cold target added : $1x10^6$	$5x10^5$	$2.5x10^5$	$1x10^5$
	A	B	D/DR				
F	1,2,	12,-,	6,8,	72*	78	81	82
C	2,26,	7,-,	1,7,	6	10	10	14
218	2,26,	15,22,	1,-,	19	14	28	28
239	3,26,	12,-,	2,-,	54	67	79	79
254	2,32,	7,40,	2,-,	5	8	13	11
280	2,3,	7,-,	1,7,	2	-1	1	1

* Results expressed as % specific ^{51}Cr release in the presence of 4 concentrations of cold target (PHA blasts).

No inhibition is seen with cells F (autologous to the responder i.e. negative control) and almost none with cells of individual n°239 ; Cell C (specific stimulator) as well as cells 254 and 280 (HLA-B 7) and 218 (HLA-B 22) are patent cold target inhibitors.

3.4. Cytotoxic clones derived from immunized patients and expressing HLA-restricted anti-H-Y specificity.

In contrast to the results obtained using as starting population in vitro primed combinations, using PBL from immunized patients (boost in vitro for 6 days by the specific cells of the immunizer followed by limiting dilution cloning), we obtain using lectin+irradiated PBL for growth promotion a large number of cytotoxic primary colonies expressing the original specificity (Table 4). None were found to proliferate against specific HLA-A2 positive male cells in a PLT assay. Large number of such colonies have been grown. Up to three successive limiting dilutions per primary colonies have been done in order to assess the clonal nature of such

cells*.

Table 4. HLA-A2 restricted lysis by 2 different primary colonies.

Clone number	E/T	Target cells (PHA blasts) A2 +ve/♂	A2 -ve/♂	A2 +ve/♀	A2 -ve/♀
C.1b.B3	16.3/1	29*	4	6	3
	8.1/1	23	3	5	1
	4/1	17	4	5	2
	2/1	17	5	1	0
	1/1	9	4	5	0
	0.5/1	8	7	1	2
C.a2	6.2/1	53	1	0	2
	3.1/1	48	1	2	-2
	1.5/1	35	1	2	-1
	0.75/1	25	0	0	-1
	0.38/1	12	1	2	-2
	0.19/1	9	1	0	-1

* Results expressed as percent of specific ^{51}Cr release.

4. DISCUSSION

From our earlier work (2, 3) we have derived a reliable technique to expand and maintain alloreactive human T cells using as growth promoter either TCGF or lectin and irradiated PBL or a combination of both. Using similar techniques we present evidences that one can easily obtain (using limiting dilution cloning of stock population of either in vitro primed or in vivo immunized cells of patients), growing colonies. We have obtained primary colonies expressing either one of the two immune reactivities tested, i.e. cytotoxic or PLT. Furthermore, within each monofunctional primary colony the immunogenetic pattern of reactivity of the colonies versus the populations was always more restricted. Such colonies can be grown to at least 50 to 100 x 10^6 functional T cells. Depending upon the starting material many more PLT than cytotoxic

*E. GOULMY, E. BLOCKLAND, B. MALISSEN, D. CHARMOT. 1980. Clones of HLA restricted male cytotoxic T cells. Production, expansion and clonal analysis. Submitted.

clones can be derived from in vitro primed cells, while no reactive PLT clones can be obtained from immunized individuals. All the T cells of the primary colonies are E rosette positive, Ig negative, HLA-A, B, C and DR positive. Furthermore, they express markers common to all T cells as well as unique markers specific of their function, as defined by xeno-monoclonal anti-T cells antibodies. They are not autonomous in the sense that they have an absolute requirement for an extrinsic growth promoter. Although these data are very encouraging, we are faced with as yet unresolved problems.

At the population level, continuously growing alloreactive T cells tend to loose after one to two months the PLT reactivity, while maintaining up to nine months their CML reactivity. However, with time, the specificity fo the cytotoxicity tends also to restrict itself.

At the clonal level, again we are faced with problems. Apart from the anti-HY HLA restricted clones, the reactivity of which were unchanged for 68 days, all the PLT reactive clones derived from in vitro primed cells, lost their specific function 15 to 20 days after the initial screening, although these cells were remarkably growing with the use of the T cells growth promoters. This is a major limitation in the ungoing progresses. We are now investigating some of the possible reasons involved in this loss of specific function. It remains to be see if long term functional clones will remain the exception. If not, such techniques will be informative only for genetics and, not for producing standardized reagents for exchange or biochemical studies.

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