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Genes and Antigens in Cancer Cells –

The Monoclonal Antibody Approach

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Genes and Antigens in Cancer Cells –

The Monoclonal Antibody Approach

Volume Editors
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Preface

Oncology in its clinical and theoretical ramifications has undergone an intimate merger with immunology in recent years. Though for quite some time, tumour immunology has existed as a discipline more by wishful thinking than by generation of hard data, the recent development of several new techniques has revolutionized the approach to malignant transformation. Modern immunology has contributed not only exciting new tools for the precise analysis and localization of molecules in whole cell, but it has also provided new concepts of gene organization and gene expression, vital to our understanding of cell differentiation. As demonstrated in the analysis of the immunoglobulin genes and their interaction with oncogenes, unexpected links have emerged between possible control mechanisms of malignant growth and genuine immunological differentiation processes.

It was on the broad field of cell differentiation that the "Deutsche Krebshilfe" decided to convocate a number of prominent scientists in order to exchange the latest information and newest ideas. Although there was a strong motivation within the "Deutsche Krebshilfe" to concentrate on applied research, the organizers were completely free to compose the final programme.

As it turned out the range of topics treated in the conference showed a considerable breadth reaching from molecular genetics and theoretical immunology to clinical applications such as radioimaging or therapy of human tumours with monoclonal antibodies.

With the title, "Genes and Antigens in Cancer Cells", it became clear that not all important points of current research could be covered. However, during the conference presentations, many start-

ing points for wider discussion were given. The conference began with addresses on central issues of current immunology. *Meurer's* contribution provided the evidence for clontypical expression of surface glycoproteins on cloned T lymphocytes. It was clear the combination of two approaches – the monoclonal antibody technique and the in vitro growth of cloned T cells – now presents the unresolved question of antigen recognition by T cells amenable to analysis.

The frequently invoked heterogeneity of tumour cells was elegantly shown by *Knapp* in an analysis on human leukemia cells with monoclonal antibodies. As demonstrated by *Stein*, the classification of Hodgkin's disease and large cell lymphoma is about to appear under a new perspective based on the presence of a hitherto unknown antigen defined by monoclonal antibody Ki-1. One of the central issues of the conference, the role of oncogenes, was discussed by *Croce*, who presented a review on his results on the effects of oncogenes when translocated to the heavy chain locus in Burkitt lymphoma cells. The possible role of human T leukemia virus in the causation of human leukemia and acquired immunodeficiency was discussed by *Gallo*. As described by *Lane* and *Mölling* monoclonal antibodies are particularly useful in defining proteins encoded by viral genes in several tumour virus systems.

The intricacies of carbohydrate chemistry on tumour cells were dealt with in presentations of *Ginsburg* and *Feizi* who showed how monoclonal antibodies can be applied for determining sequences of sugars in glycolipids and glycoproteins. One of the major glycoproteins on tumour cells is the carcinoembryonal antigen, the isolation of the messenger RNA of CEA was presented by *Zimmermann*.

A whole section of the conference was devoted to the cartography of surface antigens on human melanoma cells. *Hellström*, *Brüggen* and *Johnson* gave their view on the current state of art in this exciting field of human tumour immunology.

It is reasonable to expect that in the foreseeable future we will have a good inventory of surface molecules on this particular human tumour. A similar study on kidney carcinoma cells was presented by *Bander* from the Sloan Kettering Institute, who reported on a large library of monoclonal antibodies defining several differentiation antigens in kidney cancer cells and in normal kidney cells.

The last half day of the meeting was devoted to diagnostic and therapeutic applications of monoclonal antibodies.

New tumour markers in serum, originally defined by monoclonal antibodies on tumour cell surfaces, were presented by *Herlyn*. The recent classification of cytoskeleton proteins according to major cell differentiation lineages has opened up a new avenue to tumour typing. As demonstrated by *Osborn* monoclonal antibodies are particularly useful for typing intermediate filament proteins in tumour cells of uncertain histogenetic origin.

Vitetta gave a review on her work on therapy of mouse lymphoma tumour cells with monoclonal antibodies. *Mach* summarized his data on imaging using a variety of monoclonal antibodies. As to therapy, *Sears* presented the first data on monoclonal antibodies in colon carcinoma patients. With the outlook on the existing experimental models and the first studies in patients, it became quite clear that with the advent of monoclonal antibodies the era of a new pharmacology is about to unfold!

The organizers are greatly indebted to the "Deutsche Krebshilfe" for financial support and to several people who played a key role behind the scenes. Our thanks are particulary due to the staff of the "Deutsche Krebshilfe" whose devotion to the task and effectiveness in coping with numerous, varied arrangements did so much in assuring the final success of the conference.

Munich, December 1983 *G. Riethmüller*

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Clonal Analysis of Human Immunoregulatory and Effector T Lymphocytes in Viral Infection*

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Introduction

With the development of monoclonal antibodies to human T lymphocyte surface determinants, it has become clear that two functionally distinct human T cell subsets exist, and that these subpopulations express unique cell surface molecules. Thus, the T4+ subset provides inducer/helper function for T-T, T-B and T-macrophage interactions whereas the T8+ subset principally functions in a suppressive mode. Moreover, although both subsets of cells proliferate to alloantigen in mixed lymphocyte culture, the vast majority of cytotoxic effector function is usually detected in the T8+ population. It is important to note that the development of cytotoxicity by T8+ cells in general requires interactions with T4+ cells or their soluble products. In contrast, only a minor component of cytotoxic effector function resides within the T4+ subset and this is maximal when T4+ cells alone are sensitized in MLC [1–3].

In the face of antigenic challenge by viral, fungal, protozoan or bacterial pathogens, these cells are activated to fulfill their individual functional programs [4–11]. The complexity of cellular immune responses to viral infections has been studied both in vitro and in vivo. For example, in acute mononucleosis, both cytotoxic as well as suppressor T lymphocytes directed against virus-infected B cells are activated, presumably in order to terminate viral replication and B cell hyperreactivity [12–17]. At the same time, a specific antibody response directed at viral gene products is induced by T lymphocytes.

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Together, these mechanisms are believed to be sufficiently protective to result in cessation of clinical disease activity.

The in vitro generation of clonal T cell populations which are specific for EBV infected autologous B lymphocytes now affords the opportunity to directly study the individual T cell elements involved in the above immune responses [14, 17, 18]. Thus, in the present report we characterized a series of human T cell clones specific for autologous EBV transformed B lymphoblastoid cells with regard to immunoregulatory and effector function. In the results we demonstrate that three discrete T cell populations involved in the regulation of B cell immunoglobulin production can be defined at the clonal level. In addition, cytotoxic effector lymphocytes derived from both major human T cell subsets, T4+ and T8+, are generated in this in vitro system which exhibit dual specificity for virally encoded or induced antigens in the context of different classes of MHC gene products.

Results and Discussion

Surface Phenotypes and Function of Regulatory T Cell Clones

A series of semi-solid agar derived human T cell colonies [19, 20] generated against an autologous EBV transformed B cell line (termed Laz 509) was analyzed for their capacity to regulate Ig production by autologous B lymphocytes. To this end, 1.5×10^5 autologous peripheral blood mononuclear cells (PBMC) and 0.5×10^5 cells derived from individual T cell colonies were incubated with PWM for 7 days at 37 °C. Subsequently, culture supernatants were harvested and quantitated in a solid phase radioimmunoassay (RIA). Of 22 T cell colonies tested, 3 induced a significant increase in B cell IgG secretion, 11 strongly inhibited IgG secretion and 8 colonies had no effect. It was found that all inducer T cell clones reacted with anti-T4 whereas clones which inhibited Ig production from PBMC were either anti-T4 or anti-T8 reactive (table I). A number of the above colonies were cloned by limiting dilution technique, expanded in liquid culture and characterized with a series of monoclonal antibodies directed at T cell surface structures. Three representative immunoregulatory T cell clones termed $AT4_{II}$, $AT4_{IV}$ and $AT8_{III}$ were chosen for in depth analysis.

Table I. Distribution of regulatory T cell colonies following stimulation with autologous EBV transformed B lymphocytes

	T8+	T4+
Enhancement	0	3
Inhibition	8	3

Figure 1 indicates the surface phenotypes of $AT4_{II}$, $AT4_{IV}$ and $AT8_{III}$ as determined by indirect immunofluorescence with monoclonal antibodies and goat anti-mouse FITC on an Epics V cell sorter. As shown, all clones express the mature T cell surface glycoproteins T1, T3 and T12. In addition, each population is Ia+, a characteristic of activated T lymphocytes. Whereas $AT4_{II}$ and $AT4_{IV}$ are reactive with anti-T4 and unreactive with anti-T8, $AT8_{III}$ is reactive with anti-T8 and unreactive with anti-T4. In no case did these or other clones coexpress anti-T4 and anti-T8 reactivity.

The regulatory influence of $AT4_{II}$, $AT4_{IV}$ and $AT8_{III}$ on IgG synthesis was examined in 4 separate experiments. The latter were performed over a period of several months with consistent results indicative of the clones' functional stability. Given the observed variation in the absolute amount of IgG synthesized among individual experiments, the results are expressed as percentage of control for simplicity of comparison (100% = autologous PBMC + PWM). As shown in

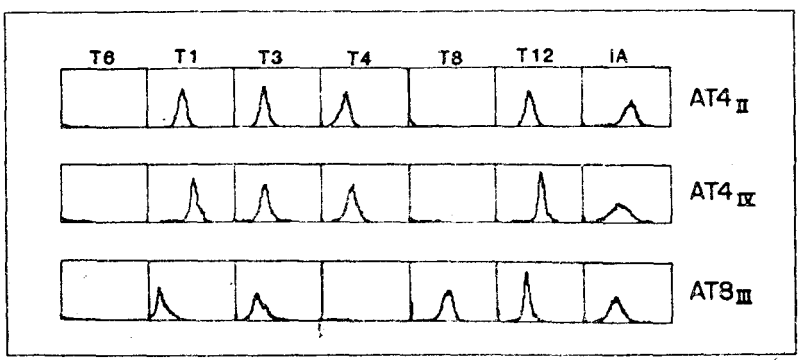

Fig. 1. Cell surface expression of differentiation antigens by clones $AT4_{II}$, $AT4_{IV}$ and $AT8_{III}$. T cell clones were incubated with saturating concentrations of one or another monoclonal antibody in ascites form and goat anti-mouse $F(ab')_2$ FITC. Analysis was performed on 10000 cells/sample employing an Epics V cell sorter.

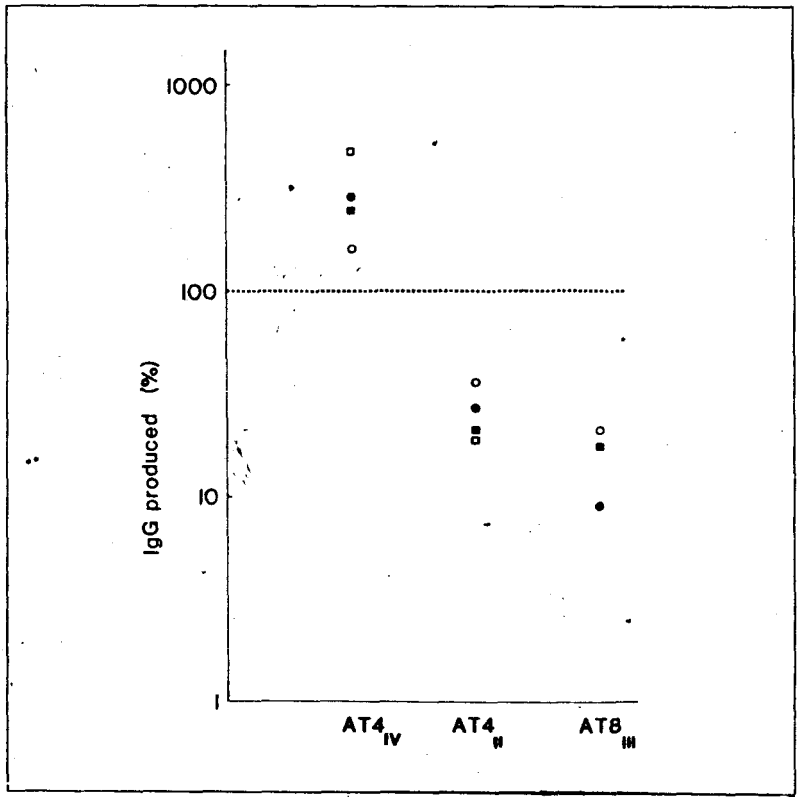

Fig. 2. Regulatory activities of clones $AT4_{II}$, $AT4_{IV}$ and $AT8_{III}$. 1.5×10^5 autologous PBMC and 0.5×10^5 cloned cells were incubated for 7 days with PWM (1:500). Supernatants were then analyzed for total IgG in a solid phase RIA. Results are given as percentage of the control: – – –, 100% (1.5×10^5 PBMC stimulated with PWM, 1:500). The various symbols (■, ●, □, ○) represent the results of four individual experiments performed over a period of several months.

figure 2, clone $AT4_{IV}$ significantly enhanced the secretion of IgG production by PBMC in each experiment performed (160–430% of control). In contrast, both $AT4_{II}$ and $AT8_{III}$ exhibited strong suppressive activity (17–35% and 9–22%, respectively). The inductive activity of $AT4_{IV}$ was due to a direct effect on B lymphocytes since this clone by itself triggered isolated B cells to produce Ig (data not shown).

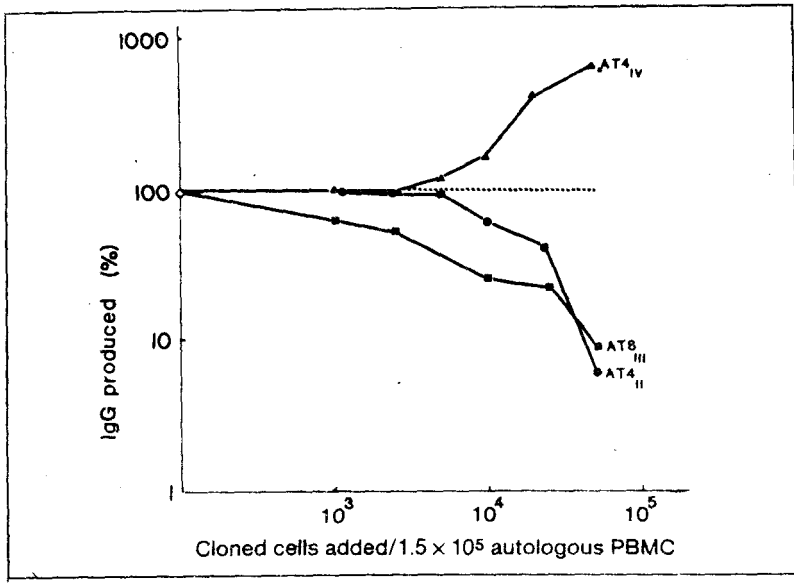

Fig. 3. Effects of varying numbers of cloned cells on IgG production. 1.5×10^5 autologous PBMC and varying numbers of $AT4_{II}$, $AT4_{IV}$ and $AT8_{III}$ cells were incubated with PWM (1:500) for 7 days. Supernatants were analyzed for IgG in a solid phase RIA. ●, $AT4_{II}$; ▲, $AT4_{IV}$; ■, $AT8_{III}$. Note that the results are expressed on a log vs. log plot.

In the above experiments, a fixed number of cloned cells had been added to each culture. We next investigated the effects on immunoregulatory function by varying the number of $AT4_{II}$, $AT4_{IV}$, and $AT8_{III}$ cells when added to a constant number of autologous PBMC (1.5×10^5/well) in the presence of PWM. As shown in figure 3, $5-10 \times 10^3$ cells from clone $AT4_{IV}$ were sufficient to provide help for IgG synthesis. Moreover, IgG production increased in a dose dependent fashion with increasing numbers of $AT4_{IV}$ cells. In a reciprocal way, the addition of increasing numbers of either $AT4_{II}$ or $AT8_{III}$ cells depressed IgG synthesis by PWM stimulated PBMC. Greater than or equal to 10^3 $AT8_{III}$ cells or $> 5 \times 10^3$ $AT4_{II}$ cells resulted in a diminution of Ig production. These dose dependent results excluded the possibility that the above differential immunoregulatory effects of these clones were due to trivial variations in culture conditions which they might have produced at a single concentration.

Dissection of Clonal Suppressor Activities

In earlier studies employing heterogeneous peripheral blood T cell populations, it was demonstrated that generation of suppression required an interaction between a radiosensitive T4+ inducer cell and a radiosensitive T8+ suppressor cell. The former was necessary to activate the latter to become a T8+ suppressor effector [21–24]. It was, therefore, of importance to determine whether $AT4_{II}$ and $AT8_{III}$ clones themselves had a direct suppressive effect on IgG synthesis or alternatively, whether they mediated suppression via additional effector T cell populations. To this end, fresh autologous T lymphocytes and fractionated T4+ and T8+ T cells were added in varying combinations to B lymphocytes with one or another T cell clone. The mix-

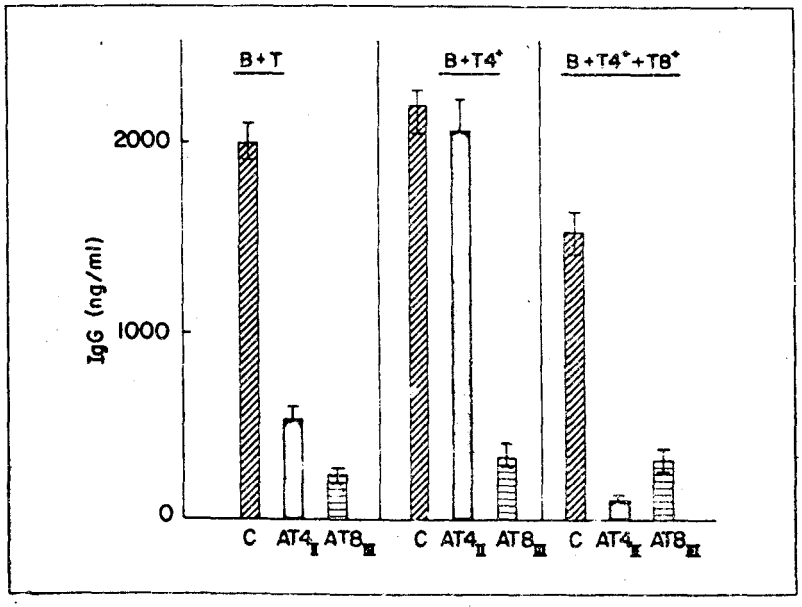

Fig. 4. Dissection of suppressor inducer and suppressor effector function. 0.5×10^5 B lymphocytes were incubated in various combinations with E+ lymphocytes (1×10^5), T4+ lymphocytes (0.5×10^5), T8+ lymphocytes (0.3×10^5) and clones $AT4_{II}$ or $AT8_{III}$ (0.5×10^5) for 7 days in the presence of PWM (1:500). Supernatants were analyzed for total IgG in a solid phase RIA.

ture was then stimulated with PWM for 7 days and IgG synthesis assayed as above. As shown in figure 4, the mixture of PWM stimulated B cells and unfractionated T cells resulted in 2000 ng/ml of IgG during the 7-day culture. Addition of either $AT4_{II}$ or $AT8_{III}$ to this mixture resulted in marked suppression of IgG production (530 and 230 ng/ml, respectively). In contrast, very different results were obtained when $AT4_{II}$ and $AT8_{III}$ cells were individually added to a combination of purified autologous T4+ T cells and B cells. Whereas $AT4_{II}$ induced little or no diminution in Ig production, $AT8_{III}$ reduced IgG production by 75%.

The above finding suggested that the suppressive effect of the $AT4_{II}$ clone was mediated via the T8+ cells present in the unfractionated T cell population. To test this possibility, $AT4_{II}$ cells were added to a mixture of autologous B cells, T4+ T cells and T8+ T cells stimulated by PWM. As shown in figure 4, addition of fresh T8+ T cells to the peripheral mononuclear mixture reconstituted the suppressive effect of the $AT4_{II}$ clone (B+T4+T8 = 96 ng/ml vs. B+T4 = 2000 ng/ml). Although not shown, irradiation of the unfractionated T cell population with 1500 rad also markedly diminished the suppressive effect of the $AT4_{II}$ cells. From these results, it would appear that the $AT8_{III}$ clone represents a suppressor effector cell whereas the $AT4_{II}$ clone, in contrast, induces a T8+ pre-suppressor cell to become a suppressor effector (i. e., inducer of suppression). In addition to emphasizing the distinct subpopulations involved in the generation of suppression, the above experiment excluded the possibility that suppression mediated by $AT4_{II}$ resulted from nonspecific cytotoxicity. If the latter were the case, then $AT4_{II}$ would have killed the autologous T4+ plus B cell mixture, resulting in reduced rather than the observed normal IgG secretion.

Regulatory Effects of Supernatants

In order to determine whether soluble factors derived from $AT4_{II}$, $AT4_{IV}$ or $AT8_{III}$ could mediate some of the clone's regulatory function, individual T cell clones were incubated at 1.5×10^6 cells/ml with an equal number of irradiated stimulator cells (Laz 509, 5000 rad) for varying periods of time. Subsequently, supernatants were harvested, added to fresh autologous PBMC in the presence or ab-

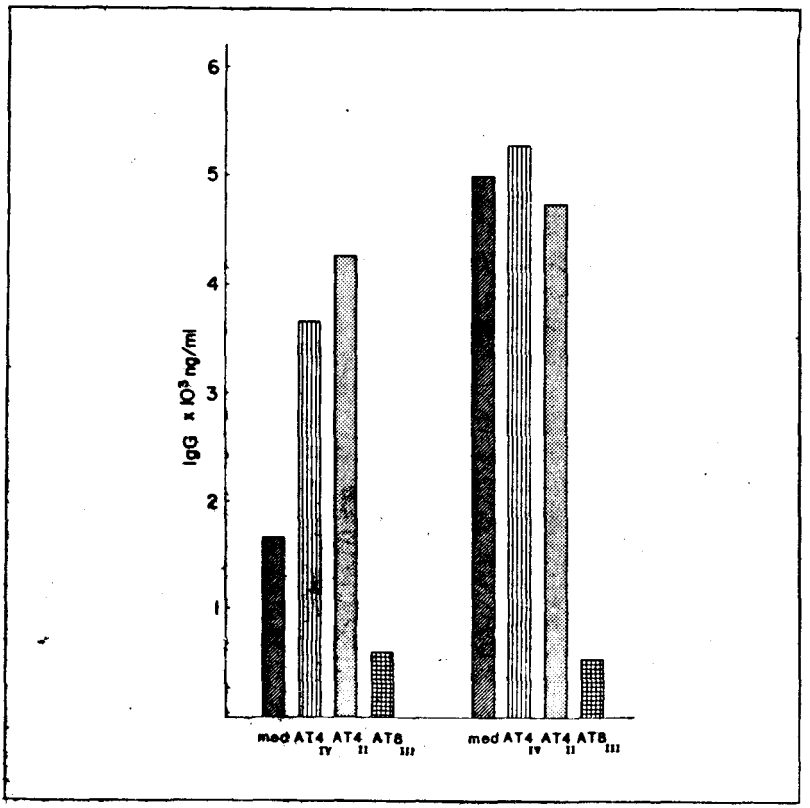

Fig. 5. Regulatory activities of supernatants derived from stimulated clones $AT4_{II}$, $AT4_{IV}$ and $AT8_{III}$. 1.5×10^5 autologous PBMC were incubated with one or another supernatant (final concentration: 20%) for 7 days without PWM (left group of columns) or with PWM (1:500) (right group of columns).
■, medium control; ▥, $AT4_{IV}$; □, $AT4_{II}$; ⊞, $AT8_{III}$.
Supernatants were analyzed for total IgG in a solid phase RIA.

sence of PWM during a 7-day culture period and then IgG secretion quantitated. Figure 5 demonstrates the results of one of a series of experiments of this type. As shown, PBMC spontaneously produce approximately 2000 ng/ml of IgG during the 7-day culture and this was enhanced to 5000 ng/ml upon PWM stimulation. Supernatants from both $AT4_{IV}$ and $AT4_{II}$ provided helper function for Ig production by the unstimulated PBMC. In contrast, the $AT8_{III}$ supernatant

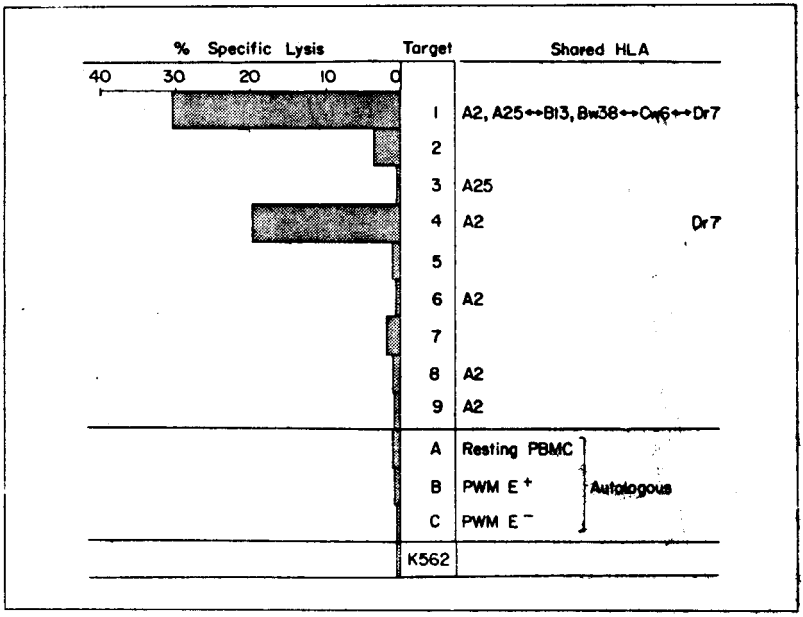

Fig. 6. Dual specificity of clone $AT4_I$ in cell mediated lympholysis. $AT4_I$ was incubated at a 15:1 E/T ratio with a panel of ^{51}Cr labelled EBV transformed HLA typed lymphoblastoid B cell lines [1–9), autologous resting and PWM activated cells and the human standard NK target, K562. The results represent mean values of triplicate cultures in a standard CML assay. Target [1] is the autologous B cell line to which $AT4_I$ had been generated.

inhibited Ig production in the same unstimulated PBMC population. In addition, $AT8_{III}$ markedly suppressed Ig production from the PWM stimulated PBMC. In contrast, supernatants from the two AT4 clones did not detectably enhance or diminish PWM stimulated Ig synthesis.

The inductive and suppressive effects of the $AT4_{IV}$ and $AT8_{III}$ supernatants corresponded to the activities that the respective clones themselves mediated upon addition to PBMC. In both cases, the activities were detectable in supernatants following 48–72 h of stimulation but could not be detected in supernatants harvested after 96 h. Similarly, the fact that the supernatants from the antigen stimulated $AT4_{II}$ clone contained a helper factor which enhanced IgG synthesis by resting PBMC was consistent with the effects mediated by the

clone itself in the absence of PWM (data not shown). In contrast, the inability of the $AT4_{II}$ supernatant, unlike the $AT4_{II}$ clone, to suppress Ig synthesis by the PWM stimulated PBMC, suggests that the suppressor inducer factor, if it existed, was not stable under these experimental circumstances.

Clonal Reactivity with JRA Sera and Anti-TQ1

Given the above findings and earlier data indicating that the T4 population could be separated into T4+JRA−TQ1− inducer and T4+JRA+TQ1+ suppression inducer subpopulations, the reactivity of the T cell clones with JRA autoantibodies (present in sera of some patients with juvenile rheumatoid arthritis) and the monoclonal antibody anti-TQ1 was determined [23, 24]. For this purpose, JRA reactivity was assessed in a complement dependent lysis assay whereas anti-TQ1 reactivity was determined by means of indirect immunofluorescence. As shown in table II, both $AT4_{II}$ and $AT8_{III}$ were reactive with JRA antisera whereas $AT4_{IV}$ was unreactive. This was of interest in light of the fact that the former two clones were involved in mediation of suppression whereas the latter was not. Moreover, table II indicates that unlike the resting PBMC population, all clones including the $AT4_{II}$ T4+ inducer of suppression were unreactive with monoclonal antibody TQ1. The finding that anti-TQ1 did not react with any of the clones tested, including $AT4_{II}$, was somewhat unexpected. One explanation for the latter finding could be that the surface expression of the TQ1 molecule was lost following T cell activation. In this regard, we have recently observed that activation of T4+TQ1+ cells in the autologous MLR results in diminished anti-TQ1 activity when analyzed sequentially.

Cytotoxic Effector Function of EBV Specific T Cell Clones

In the next series of experiments, we characterized the cytotoxic effector function of T cell clones generated in this in vitro system. Thus, a number of soft agar derived colonies were investigated for their capacity to lyse the autologous EBV transformed stimulator B cell line, Laz 509. As outlined in table III, 46 cultures expressed the