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Immunology

Involvement of Lyt-2 and L3T4 in activation of hapten-specific Lyt-2$^+$ L3T4$^+$ T-cell clones

(monoclonal antibody/major histocompatibility gene complex/T-cell antigen receptor/lymphokines)

BARBARA FAZEKAS DE ST. GROTH, PAULINE F. GALLAGHER, AND JACQUES F. A. P. MILLER

Thymus Biology Laboratory, The Walter and Eliza Hall Institute of Medical Research, Post Office, The Royal Melbourne Hospital, Victoria 3050, Australia

Contributed by Jacques F. A. P. Miller, December 2, 1985

ABSTRACT **The murine T-cell surface molecules Lyt-2 and L3T4 play a role in the activation of antigen-specific T cells. The currently accepted model for the function of these molecules proposes that Lyt-2 and L3T4 increase the overall avidity of the interaction between the T-cell antigen receptor and antigen in association with the major histocompatibility complex (MHC) molecules on the antigen-presenting cell. We have used two unusual Lyt-2$^+$ L3T4$^+$ class II MHC-restricted T-cell clones to test whether Lyt-2 can substitute for L3T4 when the T-cell antigen receptor is class II MHC-restricted. Monoclonal antibodies against L3T4 profoundly inhibited antigen-induced lymphokine production by both T-cell clones. Anti-Lyt-2 monoclonal antibody had no effect. These results strongly suggest that L3T4 and the class II-restricted T-cell antigen receptors are physically close during antigen recognition, probably as part of a multimolecular complex from which Lyt-2 is excluded. The ability of L3T4 but not Lyt-2 to participate in such a complex with class II-restricted T-cell antigen receptors may explain the striking correlation between class II restriction and L3T4 expression in the peripheral T-cell pool.**

Activation of T lymphocytes depends not only on clonally distributed T-cell antigen receptors (TcR) but also on a number of relatively invariant surface glycoproteins such as Lyt-2 and L3T4 (homologous to the human T8 and T4 antigens, respectively). In general, these two molecules are expressed in a mutually exclusive fashion on mature T cells and have therefore been used to define T-cell subsets. The vast majority of Lyt-2$^+$ cells recognize antigen in association with a product of the class I major histocompatibility complex (MHC) genes, whereas expression of L3T4 is characteristic of the class II-restricted subset of T cells (1). At the population level, most of the cytotoxic/suppressor function resides within the Lyt-2$^+$ subset, whereas helper capability is a function of the L3T4$^+$ subset. However, the correlation between T-cell function and the expression of Lyt-2 or L3T4 is weaker than that between MHC-restriction pattern and Lyt-2/L3T4 expression.

Evidence implicating Lyt-2 and L3T4 in T-cell activation comes from antibody-mediated blocking experiments. Antibodies to Lyt-2 can simultaneously inhibit cytotoxicity, proliferation, and lymphokine production by Lyt-2$^+$ T-cell clones (reviewed in refs. 2 and 3), suggesting that Lyt-2 is involved in the initial antigen-recognition event. However, there is marked variation in the degree to which anti-Lyt-2 antibodies inhibit activation of individual T-cell clones. Such variation is not a function of the amount of Lyt-2 on the T-cell surface but appears to correlate negatively with the affinity of the TcR for antigen in association with MHC molecules (antigen–MHC). Thus, secondary anti-allo-MHC responses are far more difficult to inhibit than are primary responses (2). Further support for this interpretation has been provided by the observation that anti-viral clones (from a secondary response) that crossreacted with allo-MHC in a primary response, were inhibited by anti-Lyt-2 only when recognizing the latter antigen (4). The function of the L3T4 molecule appears analogous to that of Lyt-2. Anti-L3T4 monoclonal antibodies (mAbs) block the function of some, but not all, L3T4$^+$ clones, and there is indirect evidence of a negative correlation between TcR affinity for antigen–MHC and the effectiveness of anti-L3T4 blocking (5, 6).

In the currently accepted model of antigen recognition by T cells (2), the clonally expressed TcR determines the specificity and restriction pattern of the cell. If the TcR is of low affinity, Lyt-2 or L3T4 is required only to strengthen the interaction between T cell and antigen-presenting cell (APC) in order to achieve T-cell triggering. It has been assumed that Lyt-2 is capable of binding to class I MHC and, by analogy, that L3T4 binds to class II MHC, although this is not an essential part of the model. Testing such a model is difficult, since we cannot directly measure the affinity of T-cell receptor binding to antigen–MHC nor the overall avidity of the interaction between T cell and APC. One form of the model, in which Lyt-2 and L3T4 interact with determinants on the APC independently of the TcR–antigen–MHC binding event, leads to the corollary that Lyt-2 should substitute for L3T4 in the event that both molecules are present on the T cell. Since all of the above experiments were performed with T cells expressing either Lyt-2 or L3T4 but not both, this hypothesis has not been tested. We have had the opportunity to examine this question, as we have isolated two unusual Lyt-2$^+$ L3T4$^+$ azobenzenearsonate-specific class II-restricted T-cell clones. In blocking studies using mAbs against Lyt-2 and L3T4, we tested whether Lyt-2 could replace L3T4 in stabilizing the interaction of the TcR with hapten–MHC. We found that only the mAb to L3T4 could inhibit lymphokine production by these clones, despite the fact that anti-Lyt-2 mAb also bound to the T cells. These results suggest that, during antigen recognition, a close physical association exists between the class II-restricted TcR and L3T4, and that Lyt-2 is excluded from such an association. If this is generally true for all class II-restricted TcRs, it explains why class II-restriction is found within the L3T4$^+$ rather than the Lyt-2$^+$ subset of T cells.

MATERIALS AND METHODS

Mice. Mice of the A/J, A.TL, A.TH, ASW, CBA, and C57BL/6 strains were bred at the Walter and Eliza Hall

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Abbreviations: MHC, major histocompatibility complex; APC, antigen-presenting cell(s); TcR, T-cell antigen receptor; mAb, monoclonal antibody; FACS, fluorescence-activated cell sorter; IL-2, interleukin 2 (T-cell growth factor); PSF, P cell-stimulating factor (interleukin 3); IFN, interferon; antigen–MHC, antigen in association with MHC molecules.

Institute for Medical Research and used between the ages of 8 and 16 weeks.

T-Cell Clones. The A50 and 018A lines were raised from the spleens of A/J mice skin-painted with azobenzenearsonate and restimulated weekly *in vitro* with irradiated azobenzenearsonate-coupled syngeneic spleen cells as described (7, 8). A50.3 and A50.3B are two limit-dilution clones of A50. Indirect immunofluorescence and analysis on a fluorescence-activated cell sorter (FACS) (see below) revealed that A50.3B was an Lyt-2^+ L3T4^- clone. However, every cell in the A50.3 and 018A populations expressed both Lyt-2 and L3T4. Immunofluorescence on micromanipulation clones of A50.3 and 018A confirmed that 100% were Lyt-2^+ L3T4^+. A50.3.28 and 018A.12 were chosen as representative clones for the experiments detailed below. A50.3.28 and 018A.12 have maintained steady growth rates in response to interleukin 2 (IL-2; T-cell growth factor), in the absence of added antigen or spleen cells, for more than 12 months, and their expression of Lyt-2 and L3T4 has not changed over that period. The derivation of the Lyt-2^- L3T4^+ azobenzenearsonate–Ia^k-reactive A/J clone, A3.37.4, has been described in detail elsewhere (8).

mAbs. The mAbs used in this study are listed in Table 1. In several early experiments, untreated hybridoma supernatants were used. Subsequently, antibodies were purified from hybridoma supernatants on columns of affinity-purified sheep anti-mouse immunoglobulin coupled to CNBr-activated Sepharose 4B beads (Pharmacia, Uppsala, Sweden). Antibody concentration was assessed by using an ELISA. Serial dilutions of mAbs were incubated in microtiter plates precoated with affinity-purified goat anti-mouse immunoglobulin (Antibodies, Inc.). The developing antibody was horseradish peroxidase-coupled sheep anti-mouse immunoglobulin (Tago), and the substrate used was 2,2′-azinodi(3-ethylbenzthiazolinesulfonic acid) (Sigma). Computerized linear regression analysis of logarithmically transformed absorbance data was performed as detailed for lymphokine assays (8), since the sigmoid curves from ELISA and growth factor-response assays are essentially identical.

Immunofluorescence. Cells were stained as described by Scollay and Shortman (16) with an affinity-purified sheep anti-mouse immunoglobulin, fluorescein isothiocyanate-conjugated (Silenus Labs, Melbourne, Australia) as the second-stage antibody. Analysis was performed with a Becton-Dickinson FACS II (16). Mean fluorescence channel refers to the weighted mean channel number of the sample after conversion of channel numbers to a linear scale. Percent maximum fluorescence was calculated for each sample after subtraction of the background mean fluorescence in the absence of a first-stage antibody.

Stimulation of T-Cell Clones. T-cell clones were stimulated by hapten-coupled irradiated spleen cells in RPMI 1640 medium supplemented with 1% fetal calf serum as described (8). Final cell numbers were 5×10^4 T cells and 10^6 APC in 200 μl. Monoclonal antibodies were added to T-cell clones at the same time as APC.

Lymphokine Assays. IL-2 was assayed by [^{3}H]thymidine incorporation of the IL-2-dependent CTLL line as described by Gillis *et al.* (17). The lymphokine P cell-stimulating factor (PSF, also known as interleukin 3) was assayed by [^{3}H]thymidine incorporation of the PSF-dependent R6-X E4.8.9 clone as described (8). Computerized linear regression analysis of logarithmically transformed data from the IL-2 and PSF assays was performed as detailed (8). Interferon (IFN) was assayed by inhibition of the cytopathic effect of Semliki forest virus on L cells in a microtiter assay as described (8). We have shown that IFN released by T-cell clones in response to specific antigens is of the γ subclass.

Table 1. mAbs used in these experiments

mAb	Ligand	Species	Ig subclass	Ref.
GK1.5	L3T4	Rat	IgG2b	5
H129.19	L3T4	Rat	IgG2a	9
53-6.7	Lyt-2	Rat	IgG2a	10
D9	Lyt-2.2	Mouse	IgG2a	*
331-58.1	Lyt-2.2	Mouse	IgG2b	11
19/178	Lyt-2.2	Mouse	IgG2a	12
H59-101.7	Lyt-2	Rat	ND	3
30-H12	Thy-1.2	Rat	IgG2b	10
53-7.3	Lyt-1	Rat	IgG2a	10
11-4.1	H-$2K^k$	Mouse	IgG2a	13
10-2.16	Ia^k	Mouse	IgG2b	13
PC61	IL-2 receptor	Rat	ND	14
M1/69	Heat-stable antigen	Rat	IgG2b	15

ND, not determined.

*Raised in this laboratory from a fusion of CBA spleen cells immune to the 018A cell line. The strain and tissue distribution is that expected for Lyt-2.2, and immunoprecipitation revealed a disulfide-bonded heterodimer of 30–38 kDa.

RESULTS

Characterization of T-Cell Clones by Immunofluorescence. FACS analysis of T-cell clones 018A.12 and A50.3.28 (Fig. 1) revealed that, unlike the majority of T-cell clones studied in our laboratory, every cell expressed both L3T4 and Lyt-2. A3.37.4 (Lyt-2^- L3T4^+) and A50.3B(Lyt-2^+ L3T4^-) were included as controls. Fc fragment receptor-mediated antibody binding by 018A.12 and A50.3.28 was considered very unlikely because they failed to bind several other antibodies, including M1/69 (anti-heat-stable-antigen), 10-2.16 (anti-

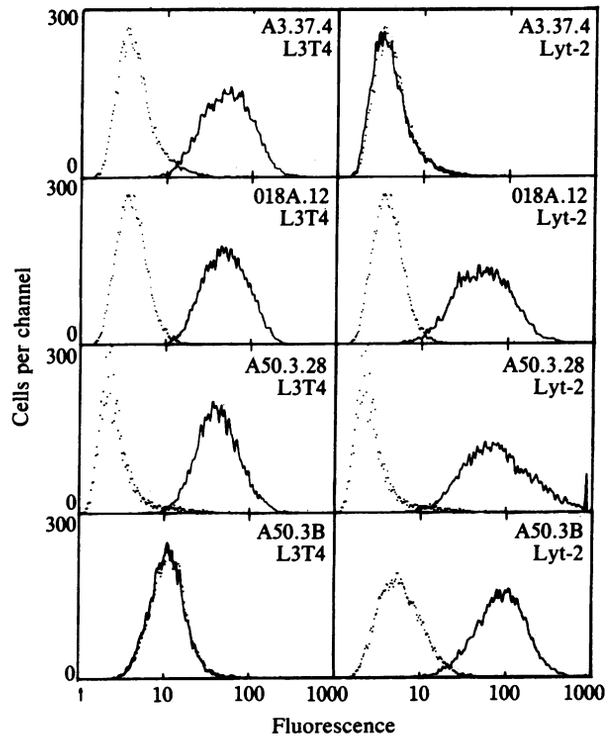

FIG. 1. Surface expression of Lyt-2 and L3T4 on T-cell clones analyzed by FACS. Cells were stained with anti-Lyt-2 (mAb 53-6.7) or anti-L3T4 (mAb GK1.5), followed by fluorescein isothiocyanate-coupled sheep anti-mouse immunoglobulin (solid lines). Dotted lines represent the negative control profile obtained with the second-layer mAb alone.

Ia^k), and the second-layer antibody (fluorescein isothiocyanate-conjugated sheep anti-mouse immunoglobulin). Moreover, five independently developed anti-Lyt-2 mAbs (detailed in Table 1) bound strongly to both 018A.12 and A50.3.28 (data not shown). Since Lyt-2^+ L3T4^+ T cells have previously been detected only in the thymic cortex, the surface phenotypes of 018A.12, A50.3.28, and A/J thymocytes (85% of which are cortical cells) were compared. In contrast to cortical thymocytes, which expressed heat stable antigen, high Thy-1, and low H-2, 018A.12 and A50.3.28 resembled medullary thymic and peripheral T cells in expressing low-to-medium Thy-1 and high H-2 and in being negative for heat-stable antigen. They also expressed the IL-2 receptor constitutively.

Functional Characterization of Lyt-2^+ L3T4^+ T-Cell Clones. Both 018.12 and A50.3.28 released IL-2, PSF, and IFN-γ in response to specific antigen (azobenzenearsonate) in association with class II MHC (Ia^k) products (Tables 2 and 3). In numerous experiments, these clones failed to demonstrate any killing of a range of azobenzenearsonate-coupled Ia^{k+} targets (data not shown).

Inhibition of Lymphokine Production by mAbs. The ability of hybridoma supernatants GK1.5 and 53-6.7 to inhibit the antigen-induced function of A50.3B and 018A.12 was tested in the experiment shown in Fig. 2. T-cell supernatants were assayed for PSF and IFN, but since A50.3B made too little PSF to give useful results, data for IFN are given. Whereas 53-6.7 was highly effective in inhibiting A50.3B (Lyt-2^+ L3T4^-), it failed to inhibit 018A.12 significantly, even at the highest concentration. Conversely, GK1.5 had no effect on A50.3B but profoundly inhibited 018A.12.

In order to ensure that saturating amounts of mAb were being added to the Lyt-2^+ L3T4^+ clones during inhibition experiments, we purified the mAbs and measured, in parallel, their ability to bind to, and to inhibit the function of, 018A.12 and A50.3.28. Since GK1.5 (IgG2b) and 53-6.7 (IgG2a) are of different Ig subclasses, a second anti-L3T4 mAb, H129.19 (IgG2a), was included. Antibodies against Thy-1 and Lyt-1 were used as controls for nonspecific inhibition at high antibody concentrations. The amount of Ig in each preparation, including two separate preparations of GK1.5 as internal controls, was quantitated in an ELISA. Surface binding of mAb to 018A.12 was measured by indirect immunofluorescence followed by FACS analysis. Plots of logarithmic antibody dilution vs. mean fluorescence channel (plotted as a percentage of the maximum fluorescence for that antibody) (Fig. 3) gave essentially parallel curves (except for 53-7.3), which allowed comparison of different antibody preparations. We chose 10% maximal fluorescence arbitrarily and calculated the concentration of antibody (ng/ml) giving this level of surface binding (Table 4). Fig. 4 *Left* shows mAb inhibition of PSF production by 018A.12 in response to azobenzenearsonate-coupled A/J spleen cells. Once again we chose an arbitrary horizontal intercept to analyze the relative efficiencies of inhibition of the various mAbs and calculated the concentration of antibody required to inhibit PSF production to 0.1 unit/ml. The results are summarized in Table 4. H129.19 and the two preparations of GK1.5 gave remarkably similar ratios of the concentration required to produce inhibition/concentration required to produce binding. 53-7.3 and 30-H12 were less efficient at inhibition by a factor of >300. 53-6.7 was less efficient by a factor of >2500, failing to inhibit at concentrations of >7.4 mg/ml, compared with profound inhibition by GK1.5 at <0.27 ng/ml and H129.19 at 2.5 ng/ml. The subclass of antibody had no effect on the ultimate outcome.

Table 2. Antigen specificity of lymphokine production by 018A.12

A/J splenic APC	Lymphokine, units/ml PSF	IFN	IL-2
ABA-coupled	64	256	5.3
TNP-coupled	<0.1	<4	<0.1
Not coupled	<0.1	<4	<0.1
No APC	<0.1	<4	<0.1

ABA, azobenzenearsonate; TNP, trinitrophenyl.

Table 3. MHC restriction of PSF production by 018A.12 and A50.3.28

ABA-coupled APC*	PSF, units/ml 018A.12	A50.3.28	MHC shared†
A/J	7.0	36	KID
A.TL	9.9	25	-ID
A.TH	<0.1	<0.1	--D
ASW	<0.1	<0.1	---
CBA	17	51	KI-

*Responses to nonhaptenated spleen cells of all five mouse strains were <0.1 unit/ml of PSF. ABA, azobenzenearsonate.
†KID refer to regions of the MHC genes.

Fig. 4 *Right* shows similar antibody inhibition curves for A50.3.28. Addition of dilutions of 53-6.7 did not modify the inhibition pattern seen with GK1.5 alone. Since 53-6.7 failed to inhibit either 018A.12 or A50.3.28, despite efficient inhibition of the Lyt-2^+ L3T4^- clone, A50.3B (Fig. 2), we performed inhibition studies with the four other anti-Lyt-2 mAbs described in Table 1 and previously shown to bind strongly to 018A.12 and A50.3.28. All four mAbs failed to inhibit lymphokine production by 018A.12 and A50.3.28 at even the highest antibody concentrations (>15 μg/ml) despite giving 10% maximal fluorescence values for both 018A.12 and A50.3.28 in the range of 5–20 ng/ml (data not shown).

DISCUSSION

Our understanding of the surface molecular events leading to T-cell activation has been hampered not only by difficulties in characterization and purification of the molecules involved but also because triggering events appear to be dependent upon complex interactions between arrays of molecules on two opposed cell surfaces. Therefore, we have had to rely on

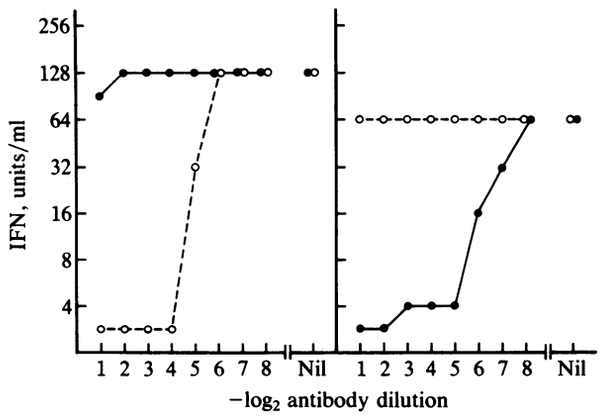

FIG. 2. Inhibition of T-cell function by mAbs to Lyt-2 and L3T4. T-cell clones 018A.12 (Lyt-2^+ L3T4^+) (*Left*) and A50.3B (Lyt-2^+ L3T4^-) (*Right*) were stimulated with azobenzenearsonate-coupled syngeneic spleen cells in the presence of various dilutions of culture supernatants from the hybridomas 53-6.7 (●) and GK1.5 (○). After 18 hr, the supernatants were assayed for IFN (*Left* and *Right*) and PSF (data not shown).

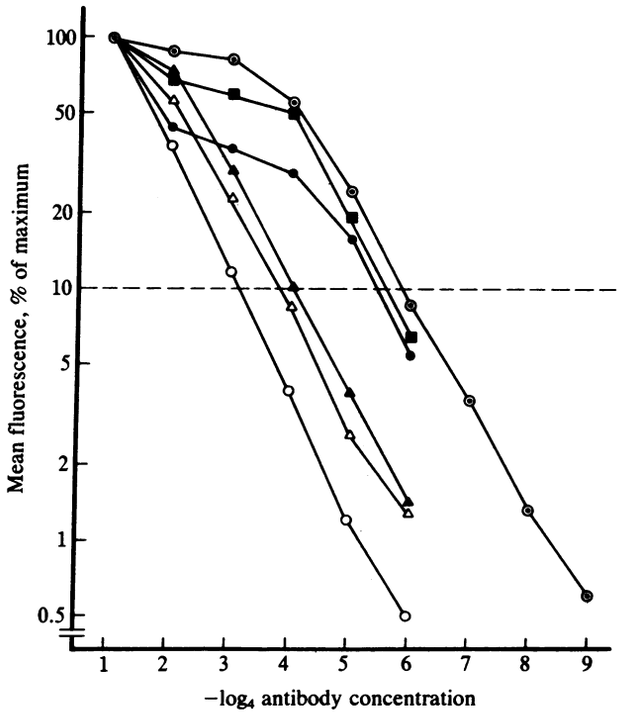

FIG. 3. Binding of mAbs to 018A.12. Cells were stained with mAbs, followed by fluorescein isothiocyanate-conjugated sheep anti-mouse Ig and analyzed by FACS. Mean channel fluorescence was calculated for each sample and was then expressed, after background subtraction, as a percentage of the maximum (plateau value) binding for that mAb; mAbs used were as follows: GK1.5, preparation 1 (○); GK1.5, preparation 2 (◎); H129.19 (△); 30-H.12 (▲); 53-7.3 (●); and 53-6.7 (■).

indirect evidence derived mainly from experiments in which antibodies stimulated or inhibited function. Only one class of clone-specific receptor molecule has so far been identified as the antigen recognition unit of T cells (18). mAbs to the TcR can either stimulate or inhibit T-cell function depending on whether they are presented as a surface array or as soluble molecules (19), suggesting that complexing of TcRs is fundamental to the triggering event. The evidence implicating Lyt-2 and L3T4 in activation of T cells is based on inhibition of antigen-induced function by antibodies to these molecules (1–6). Experiments by MacDonald *et al.* (2, 4) have suggested that variation in the degree of inhibition by anti-Lyt-2 is a

Table 4. Inhibition of 018A.12 by mAbs

mAb	Ligand	10% maximal binding,* ng/ml	Inhibition,† ng/ml	Inhibition/ binding ratio‡
GK1.5 (1)§	L3T4	0.51	0.14	0.27
GK1.5 (2)§	L3T4	0.68	<0.27	<0.40
H129.19	L3T4	7.4	2.5	0.33
53-7.6	Lyt-1	3.9	370	95
30-H12	Thy-1	4.4	>300	>69
53-6.7	Lyt-2	12	>7400	>590

*Dilutions giving 10% mean fluorescence (calculated from the horizontal intercept of Fig. 3) were converted to antibody concentrations on the basis of ELISA data (not shown).
†Dilutions inhibiting PSF production to 0.1 unit/ml (Fig. 4 *Left*) were converted to antibody concentrations on the basis of ELISA data.
‡Ratio of antibody concentration required to inhibit PSF production and antibody concentration required to give 10% maximal fluorescence.
§Two separate preparations of GK1.5 included as an internal control for the binding and inhibition assays.

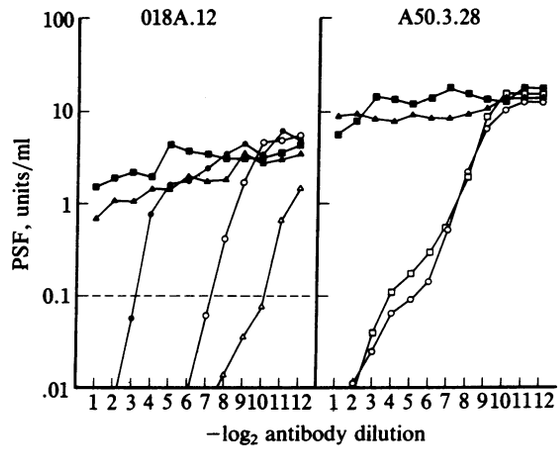

FIG. 4. Inhibition of the function of Lyt-2$^+$ L3T4$^+$ T-cell clones by mAbs. T-cell clones 018A.12 and A50.3.28 were stimulated with azobenzenearsonate-coupled A/J spleen cells in the presence of various dilutions of purified monoclonal antibodies; after 18 hr the supernatants were assayed for PSF. The antibodies used were as follows: GK1.5, preparation 1 (preparation 2 produced such profound inhibition that no PSF was detectable even at a dilution of 1:8192) (○); H129.19 (△); 30-H.12 (▲); 53-7.3 (●); 53-6.7 (■); and 53-6.7 plus GK1.5, preparation (1) (□).

function of the affinity of the interaction between the TcR and its ligand. The effect of anti-L3T4 mAb on L3T4$^+$ cells appears analogous to that of anti-Lyt-2 on the Lyt-2$^+$ subset (1). The proposed ligands for Lyt-2 and L3T4 are class I and II MHC, respectively. Further indirect evidence concerning the ability of L3T4 to interact with several different class II MHC haplotypes has recently been provided (20), although it does not appear to bind to any highly conserved monomorphic MHC determinants (21).

Since Lyt-2 and L3T4 appear to fulfill equivalent roles in Lyt-2$^+$ and L3T4$^+$ T cells, the simplest interpretation of the above model predicts that, if both Lyt-2 and L3T4 were present on the same T cell, Lyt-2 would substitute for L3T4 during antigen recognition. Our results do not support this prediction. Despite clear evidence of expression of both Lyt-2 and L3T4 by these two unusual clones, mAbs to L3T4 alone were able to inhibit lymphokine production. Had Lyt-2 substituted for L3T4 in stabilizing the TcR–antigen–MHC complex, inhibition should have required both anti-Lyt-2 and anti-L3T4. The failure of the five anti-Lyt-2 mAbs to inhibit lymphokine production suggests that the combined binding strength of L3T4 and TcR is sufficient to provide the avidity necessary for activation, whether or not Lyt-2 also contributes. The converse is not true. When the contribution of L3T4 is eliminated by mAb blockage, the avidity of the TcR plus Lyt-2 is insufficient to trigger the cell. To explain this asymmetry, we propose that L3T4 and the TcR must be relatively close together on the T-cell membrane. Since there is no evidence of coprecipitation of L3T4 and the TcR either before or after crosslinking of unstimulated T cells (22), we must propose that such a complex forms only at the time of antigen binding.

The above interpretation rests heavily upon the assumption that the Lyt-2 molecules of clones 018A.12 and A50.3.28 are capable of functioning normally. Since we have no direct assay of their ability to stabilize an appropriate TcR–antigen–MHC interaction, we must rely on characterization of the molecules themselves. Immunoprecipitation has revealed that the molecular weights of the Lyt-2/3 dimer on 018A.12 and A50.3.28 are within the range expected for functional Lyt-2-dependent T-cell clones (unpublished data). Immunoblot-hybridization analysis of RNA from the two clones has confirmed the presence of the two Lyt-2 mRNA species

present in normal spleen and thymus (unpublished data; ref. 23). Further molecular studies may reveal why the *Lyt-2* and *Lyt-3* genes are expressed in these Lyt-2$^+$ L3T4$^+$ clones. We have preliminary evidence from class II-directed mixed lymphocyte cultures that such double expression is not uncommon after activation *in vitro*. Double expression has also been reported after mitogenic stimulation of human peripheral T cells (24), but no functional studies were performed.

An intriguing possibility raised by the results presented here is the general theory that, during antigen recognition, L3T4 forms a physical association with class II-restricted TcRs, whereas Lyt-2 associates only with class I-restricted TcRs. There is a good deal of indirect evidence supporting such a contention. A striking correlation is seen between L3T4 expression and class II MHC restriction and, conversely, between Lyt-2 expression and class I restriction (1). Although there is no evidence that class I- and class II-restricted T cells use different TcR gene subsets, the failure to find any TcRs displaying both class I and class II MHC restriction patterns, despite numerous examples of cross-reactions within each class (e.g., see ref. 25), suggests that, at the protein level, the two sets of TcRs differ substantially. This may result from selection of TcRs with particular specificities once random gene rearrangement has taken place. Similarly, the two sets of TcRs may differ in their ability to form close physical associations with molecules such as Lyt-2 and L3T4 during antigen recognition. Such differences would then allow surface expression of either Lyt-2 or L3T4 to be selected during ontogeny on the basis of the ability to form an effective complex with a particular TcR. An alternative possibility is that Lyt-2 and L3T4 bind to class I and II MHC, respectively, in order to facilitate the formation of local aggregates of MHC molecules on the APC surface, thus increasing the overall avidity of the TcR–antigen–MHC interaction. In this second model, TcR and corecognition molecules are linked not by any intrinsic affinity for each other but by their ability to recognize different epitopes on the same MHC molecule.

The final outcome of the two processes postulated above is the same: a complex consisting of L3T4, class II-restricted TcR, antigen, and class II MHC. In both models, TcRs of high affinity can form simpler complexes of TcR plus antigen–MHC. Cells with such TcRs do not require L3T4 and hence are under no selective pressure to express the appropriate member of the Lyt-2/L3T4 pair. This situation may apply to many of the Lyt-2$^+$ L3T4$^-$ class II-restricted T-cell clones documented in the literature (9) in which Lyt-2 appears to have no function. In other cases, inhibition studies with anti-Lyt-2 have not been performed (26). However, there does appear to be evidence of functional L3T4 in an unusual class I-restricted hybridoma (20). This exception is difficult to explain in terms of the two hypotheses outlined above. However, it could be accommodated in the first model if steric constraints were to prevent only the vast majority of class I-restricted TcRs from forming effective complexes with L3T4. Recent evidence of positive selection of L3T4$^+$ T cells on thymic Ia$^+$ APC (27) suggests a site for coselection of L3T4 and class II-restricted TcRs in the thymus. Since such thymic APC also express class I MHC, Lyt-2$^+$ class II-restricted and L3T4$^+$ class I-restricted T cells might also be selected in rare instances. On the basis of the experimental evidence provided in this report, we suggest that physical constraints usually exist to prevent the association, and therefore coselection, of Lyt-2 and class II-restricted TcRs during T-cell maturation.

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