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Regulation of LFA-1-mediated T cell adhesion by CD4*

Heterotypic adhesion of T lymphocytes to monocytes, B lymphocytes, or other target cells is mainly mediated by LFA-1 and CD2 molecules. Low-affinity binding of resting T cells can be transiently up-regulated by cross-linking of CD3. We have previously found that binding of specific ligands to CD4 can down-regulate adhesion of resting T cells to B cells. We now show that the enhanced adhesiveness of $CD4^+$ T cells induced by CD3 cross-linking using plastic-bound anti-CD3 antibody can also be inhibited by several CD4 ligands, *i.e.* anti-CD4 antibodies, the gp160 env protein of human immunodeficiency virus, as well as by putative CD4 ligands, *i.e.* synthetic peptides analogous to the gp160-binding site to CD4 (positions 418–434 and 449–464) and a 12-mer synthetic peptide (DR-12) analogous to positions 35–46 of HLA class II β subunit and including the highly conserved Arg-Phe-Asp-Ser (RFDS) sequence. After CD3 cross-linking, maximal binding of T cells to HLA class II-positive and -negative B cells was similar, although binding to HLA class II-negative B cells was more prolonged. T cells that were passively induced to up-regulate adhesion by binding of a CD11a-specific antibody, NKIL16, known to enhance LFA-1-dependent adhesiveness, were less sensitive to the inhibitory effect of the DR-12 peptide, whereas the inhibitory effects of gp160 were preserved. The kinetics of adhesion of NKIL16-pretreated T cells was not influenced by HLA class II expression at the B cell surface. Together, these results strongly suggest that CD4-HLA class II interaction may down-regulate low-affinity adhesion of resting T cells and, to some extent, high-affinity adhesion of T cells actively induced by CD3 cross-linking but not passively induced by an anti-CD11a antibody.

1 Introduction

The antigen-independent heterotypic adhesion of T cells to monocytes, B cells or other target cells is mainly mediated by two independent molecular pathways, *i.e.* LFA-1 (CD11a-CD18) binding to ICAM-1 (CD54) or ICAM-2 ligands and CD2 binding to LFA-3 (LCD58; [1–3]). Such adhesion processes are required for T cell activation and effector functions. This has been demonstrated in blocking experiments using specific mAb [4–6] and showing altered binding and functions of T cells defective in LFA-1 expression [6–8], as well as poor binding and cytotoxic function of T cells towards $ICAM\text{-}1^-$ $LFA\text{-}3^-$ Burkitt B cells [9]. Finally, transfection of murine fibroblasts with ICAM-1 or LFA-3, in association with MHC molecules, leads to optimal binding and activation of specific T cells [10–12]. The LFA-1- and CD2-dependent low-affinity binding of T cells can be up-regulated when expression of adhesion molecules (LFA-1 and ICAM-1) is increased following the secretion of IL 1, IFN-γ and TNF [3, 13]. This event is secondary to T cell activation and can thus not account for mediating early T cell adhesion associated with T cell activation [1, 2]. Recently, Dustin and Springer [14] and Keizer et al. [15] demonstrated that CD3 cross-linking may result in an early up-regulation of T cell adhesion that relies on a transient increase in the affinity of LFA-1 for its ligand [16, 17]. This event is thought to be mediated by a conformational change related to PKC phosphorylation of LFA-1 or of an associated protein [14, 17]. A more prolonged up-regulation of T cell adhesion can also be induced by an LFA-1-specific antibody, NKIL16, that may induce homotypic cell agregation [15].

We have previously provided experimental evidence supporting the hypothesis that the CD4-MHC class II interaction could down-regulate the low-affinity binding of resting $CD4^+$ T cells to B cells ([6], Mazerolles et al., *Hum. Immunol.*, in press). Indeed, resting $CD4^+$ T cells show similar maximal binding to MHC class II-positive and -negative B cells, although binding to class II^+ B cells is transient whereas binding to class II^- B cells is prolonged for at least 20 min. Finally, anti-CD4 antibodies and the gp160 envelope protein from HIV (which binds to CD4; Corado et al., submitted), as well as peptides encompassing the gp160-binding site to CD4 [18] and peptides analogous to the 35–46 sequence of MHC class II β chains and containing the highly preserved RFDS sequence [19], all inhibit $CD4^+$ T cell-B cell adhesion. This suggests that CD4-MHC class II interaction, possibly mediated in part by the MHC class II RFDS sequence, and gp160 binding to CD4, can down-regulate antigen-independent adhesion of $CD4^+$ T cells.

In this study, we have determined whether CD4 is involved in the regulation of the high-affinity binding of $CD4^+$ T cells previously induced by CD3 cross-linking or NKIL16 (anti-CD11a) antibody binding.

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2 Materials and methods

2.1 Antibodies and peptides

The following mAb were used in the adhesion assays: NKIL16 (IgG_2a, anti-CD11a LFA-1, culture SN [15]), 25.3 (IgG_1, anti-CD11a, LFA-1, D. Olive, Marseille, France; [20]), TS1.18 (IgG_1, anti-LFA-1 β subunit [CD18], T. Springer, Boston, MA; [21], OKT3, OKT4a (IgG_2a, anti-CD3, anti-CD4, respectively, Orthodiagnostic, Raritan, NJ), 84H10 (IgG_1, anti-ICAM-1 (CD54); D. Olive; [22], BC18 (IgG_2a, anti-CD2, C. Anasetti, Seattle, WA), IOT8 (IgG_1, anti-CD8, Immunotech, Marseille, France), ALB9, IOT7 (IgG_1, IgG_2a, anti-CD24, anti-CD7 respectively, Immunotech), Leu-1 (IgG_2a, anti-CD5, Becton Dickinson, Mountain View, CA).

DR-12 peptide (EEYVRFDSDVGE) is analogous to residues 35 to 46 from the β_1 domain of HLA-DR1.2 [19]. DR43-54 peptide (DVGEYRAVTELG) is analogous to residues 43 to 54 from the β_1 domain of HLA DR1.2 class I 31–42 peptide (TQFVRFDSDAAS) is analogous to residues 31 to 42 from the α_1 domain of HLA class I A2. DR12 variant peptides were also used, *i.e.* DR12 V1 (EEYVRDSDVGE), DR12 V2 (EEYRGDSDVGE), DR12 V4 (EEYRFESDVGE). All were synthesized by Neosystem (Strasbourg, France) according to the solid-phase synthesis method of Merrifield, and further purified by HPLC, resulting in >96% purity, as shown by HPLC analysis. Peptide sequences were validated by amino acid analysis of the purified preparations.

The gp160 (env; M. Kaczorek, Pasteur-Vaccins, Val de Reuil, France), was secreted by BHK-21 cells after infection with a recombinant vaccinia virus (W-1163) expressing a complete env glycoprotein from the HIV-1 BRU isolate [23] and purified (Pasteur-Vaccins) from the culture SN by means of DEAE ion-exchange chromatography, lectin affinity chromatography and gel filtration chromatography, under sterile conditions. Purity was at least 90% as determined by reverse-phase HPLC and Western blot analysis. Peptides : 302–324 (TRPNNNTRKSIRIQRGPGRAFVT), 418–434 (STEGSNNTEGSDTITLP), 449–464 (KAKYAPPISGQIRCSS) derived from the gp160 sequence of the HIV-1 BRU isolate were synthesized by Neosystem using the solid-phase synthesis method of Merrifield and further purified (to >85% purity) using HPLC. Peptides sequences were validated by amino acid analysis of the purified preparations. The soluble CD4 given by Dr. A. Truneh (Smith Kline and French Laboratory, Swedeland, PA) was obtained by recombinant DNA technology. This soluble CD4 retains the structural and biological properties of CD4 on the cell surface.

2.2 Cell preparations

PBMC from healthy adults were isolated using Ficoll Hypaque (Pharmacia, Uppsala, Sweden) centrifugation. $CD4^+$ T lymphocytes were isolated from PBMC by a two-step procedure, *i.e.* neuraminidase-treated RBC rosetting, followed by negative selection by panning using a CD8-specific mAb (BC8, J.W. Wijdenes, Besançon, France; 1.5 μg/10^6 cells) and an goat anti-mouse Ig antibody (GaMIgG, Biomaker, Rehovot, Israel). $CD4^+$, T cell preparations contained <2% $CD8^+$ T cells. $CD4^+$ T cells were also purified from PBMC of a patient with leukocyte adhesion deficiency (LAD) and very low leukocyte expression of LFA-1 [5]. EBV-induced B cell lines were prepared from PBMC of healthy individuals and from immunodeficient patients with an inherited deficiency in HLA class II expression molecules [24]. Mutant HLA class II^- B cells (RJ225) produced by Accolla et al. [25] were cultured as described. In some experiments, $CD4^+$ T lymphocytes were preincubated with NKIL16 at 37 °C for 15 min, then washed and incubated with EBV-B cell lines for conjugate formation.

$CD4^+$ T lymphocytes were also activated by anti-CD3 antibody (OKT3), according to the method of Dustin and Springer [14] with slight modifications. Briefly, $CD4^+$ Tcell (10^7/ml) were incubated in 5% FCS for 30 min at 4 °C on petri dishes precoated with OKT3 (50 μg/ml) for 1 h at 37 °C. Unbound cells were eliminated by washing; bound cells were removed using a rubber policeman, washed and used for conjugate formation.

2.3 Immunofluorescence

Cell fluorescence intensity was evaluated by means of FCM analysis using a FACStar plus (Becton Dickinson) following revelation by specific antibodies and by a GaMIg (Nordic, Tilburg, The Netherlands).

2.4 Conjugate formation

Antigen-independent T-B conjugate formation was induced according to the method described by Onishi et al. [26]. Briefly, T and B cells (both at 2×10^6/ml) were incubated with hydroethydine (40 μg/ml, Polysciences, Warrington, PA) for 10 min at 25 °C and fluorescein diacetate sulfate (100 μg/ml, Molecular Probes Inc, Eugene, OR) for 10 min at 37 °C. After washing, 5×10^5 resting or activated Tcells were mixed with 5×10^5 B cells in a 500 μl volume of RPMI 1640 medium (Gibco Biocult, Paisley, Scotland). Preliminary experiments showed that optimal conjugate formation took place after 20 min of incubation at 37 °C. Incubation was followed by centrifugation at 250 × g for 5 min and gentle resuspension before counting. Conjugates were identified as red/green pairs of cells under fluorescence microscopy. Fluorescent cells (250–350) were counted blind in each experiment. Experiments performed in duplicate by independant observers were not significantly different. Results are expressed as the percentage of T-B conjugates relative to total T cells or as the inhibition of T-B conjugate formation : [1 – (TB/T : TB control / T control)] × 100. Statistical analysis was performed by using the Student's *t*-test.

3 Results

3.1 Up-regulation of $CD4^+$ T cell adhesion by anti-CD3 (OKT3) and anti-LFA-1 (NKIL16) antibodies

As shown in Fig. 1A, incubation of resting $CD4^+$ T lymphocytes with increasing concentrations of plastic-

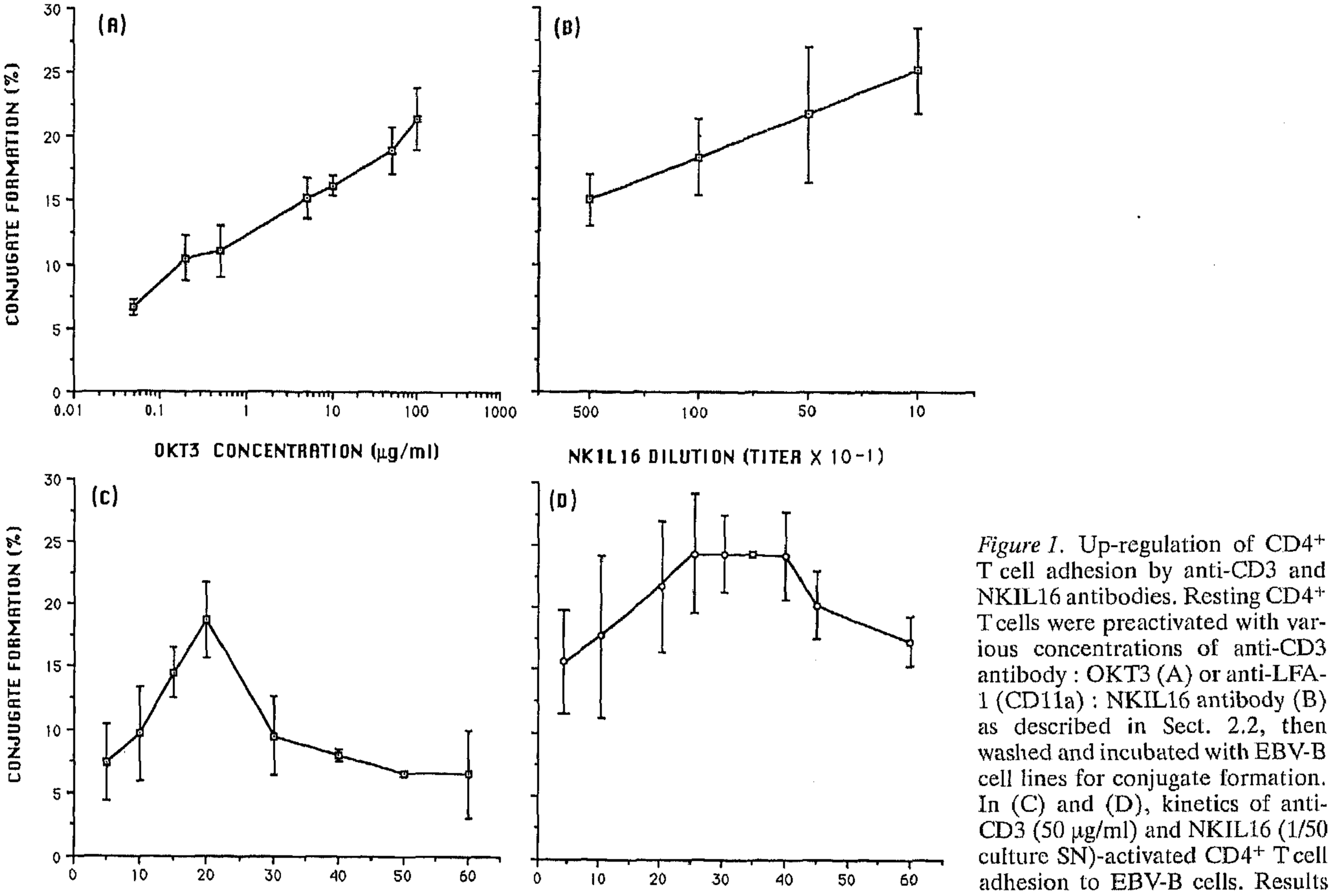

Figure 1. Up-regulation of CD4+ T cell adhesion by anti-CD3 and NKIL16 antibodies. Resting CD4+ T cells were preactivated with various concentrations of anti-CD3 antibody : OKT3 (A) or anti-LFA-1 (CD11a) : NKIL16 antibody (B) as described in Sect. 2.2, then washed and incubated with EBV-B cell lines for conjugate formation. In (C) and (D), kinetics of anti-CD3 (50 µg/ml) and NKIL16 (1/50 culture SN)-activated CD4+ T cell adhesion to EBV-B cells. Results are the mean of seven independent experiments ± 1 SD.

bound OKT3 antibody resulted in a significant increase in the percentage of T cells able to bind EBV-transformed B cells. Differences were significant with concentrations of OKT3 antibody > 0.2 µg ($p < 0.01$). As for resting CD4+ T cells, peak of conjugate formation was still observed after a 20 min incubation (Figs. 1 C and 2 A). Interestingly, CD4+ T cells that were activated by CD3 cross-linking were found to adhere only transiently to B cells, since the percentage of conjugates dropped off rapidly after 20 min.

NKIL16 is a CD11a-specific antibody, known to enhance homotypic T cell adhesion and agregation [15]. Our results indicate that a short incubation of CD4+ T cells with increasing concentrations of NKIL16 (Fig. 1 B) induces up-regulation of adhesion to B cells; peak conjugate formation occurred after 20 min of incubation, with a plateau until 45 min, after which a decrease was observed in the presence of NKIL16 antibody concentrations > 50 × 10^{-1} (Figs. 1 D and 2 A).

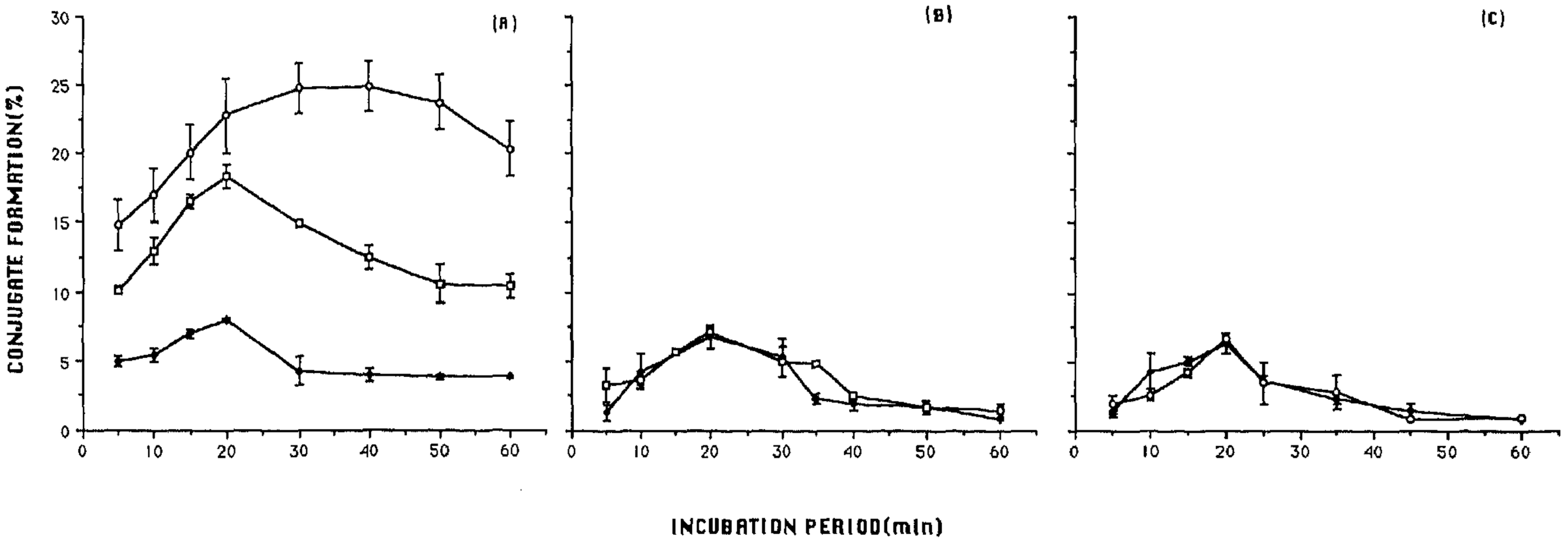

Figure 2. LFA-1dependency of anti-CD3- and NKIL16-induced up-regulation of T cell adhesion. (A) Kinetics of adhesion of resting (◆), OKT3-activated (□) and of NKIL16-activated (○) CD4+ T cells to B cells. (B) Kinetics of adhesion of resting (◆) and OKT3-preactivated LFA-1-deficient CD4+ T cells (□) to B cells. (C) Kinetics of adhesion of resting (◆) and NKIL16-preactivated LFA-1-deficient CD4+ T cells (○) to B cells. The results of one typical experiment (mean ± SD of duplicates) among five experiments are shown.

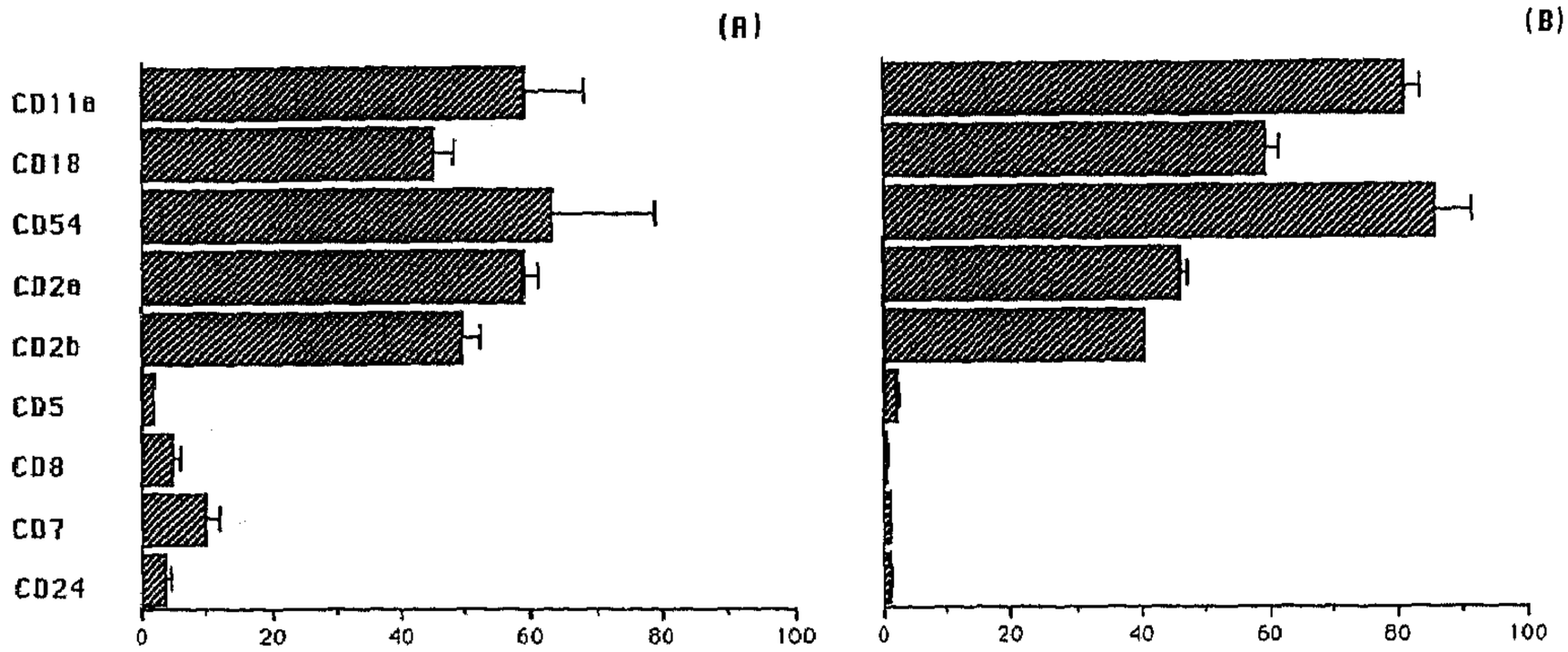

Figure 3. Inhibition of anti-CD3- and NKIL16-induced up-regulation of T cell adhesion. $CD4^+$ T cells preactivated with OKT3 at 50 μg/ml (A) or NKIL16 at 1/50 dilution (B), as described in Fig. 1, were mixed with EBV-B cell lines in the presence of the antibodies indicated (20 min, 37 °C) for conjugate formation. Anti-CD11a, -CD18, -CD54, -CD2 (CD2a : BC18; CD2b : OKT11), antibodies (1 μg/ml). Anti-CD5, -CD8, -CD7, -CD24 antibodies (10 μg/ml). The percentage of control T-B conjugates was, in (A): resting $CD4^+$ T cells: 13 ± 2.5%, preactivated $CD4^+$ T cells: 20 ± 2%; in (B): resting $CD4^+$ T cells: 10.5 ± 2%; preactivated $CD4^+$ T cells: 20.3 ± 2%. The results of one typical experiment (mean ± SD of duplicates) among five experiments are shown.

It has been found that anti-CD3-induced up-regulation of T cell adhesion is an LFA-1-dependent phenomenon, as is the case for binding of T cells to purified ICAM-1 molecules [14], that is, however, independent of the degree of membrane expression of LFA-1 [14]. This was confirmed in our $CD4^+$ T-B conjugate formation assay, since no up-regulation of adhesion of $CD11a^-$ T cells from patients LAD was observed (Fig. 2B). In addition, anti-LFA-1 (CD11a and CD18) and anti-ICAM-1 antibodies strongly inhibited anti-CD3-activated $CD4^+$ T cell adhesion (Fig. 3a). However, anti-CD2 antibodies were still able to inhibit adhesion of the same T cells, indicating that CD2 participates in the adhesion of anti-CD3-activated T cells.

As expected, the NKIL16-induced up-regulation of $CD4^+$ T cell adhesion was also found to be dependent on LFA-1 expression (Fig. 2C). Such adhesion was, however, also CD2 dependent, as shown by the partial blocking effect of anti-CD2 antibodies (Fig. 3B). Isotype-matched (IgG_1 or IgG_{2a}) anti-CD5, CD7, CD8 and CD24 antibodies had no effect on either type of activated T cell adhesion to B cells (Fig. 3).

3.2 Inhibition of anti-CD3- and NKIL16-activated $CD4^+$ T cell adhesion by anti-CD4 antibody, MHC class II-derived peptides, gp160 and gp160-derived peptides

To assess whether the CD4 molecule is involved in the adhesion of T cells preincubated with either anti-CD3 or NKL16 antibodies, we investigated the potential inhibitory activity of CD4 ligands (anti-CD4 antibody and gp160), as well as putative CD4 ligands (an MHC class II-derived peptide mimicking the 35–46 sequence of β1 and gp160-derived peptides). All these reagents have previously been shown to specifically inhibit adhesion of resting $CD4^+$ T cells ([6]; Corado et al; submitted). Initially it was checked that T cell incubation with anti-CD3 or NKIL16 antibodies did not significantly alter CD4 expression compared to resting cells (data not shown).

As shown in Fig. 4, the anti-CD4 antibody OKT4a was able to inhibit both anti-CD3 and NKIL16 activated T cell adhesion and adhesion of resting $CD4^+$ T cells [6]. In addition, this antibody was equally effective on the adhe-

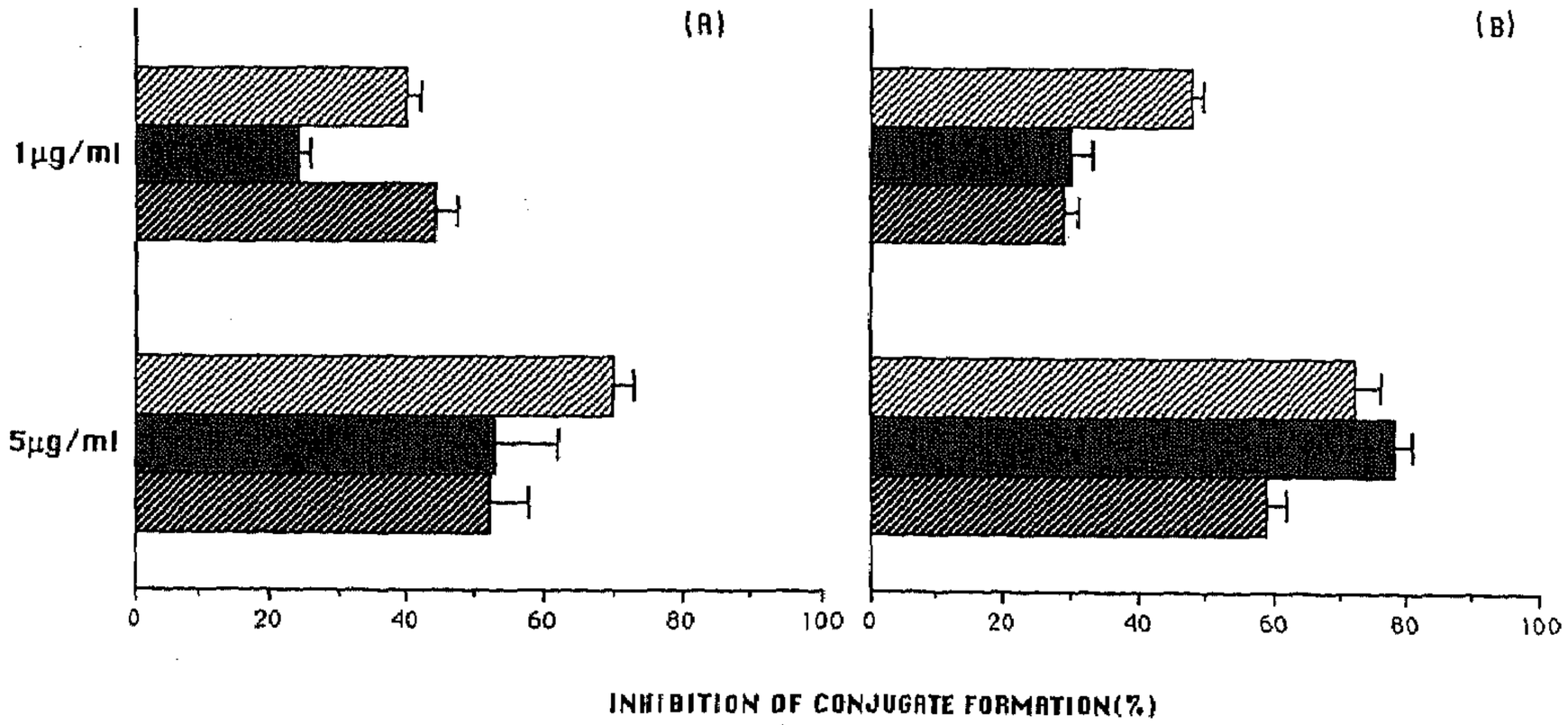

Figure 4. Inhibition by anti-CD4 antibody (OKT4A) of the $CD4^+$ T cell adhesion to MHC class II^+ and MHC class II^- B cells. (A): Adhesion to MHC class II^+ B cells. (B): Adhesion to MHC class II^- B cells. $CD4^+$ T cells were either resting (▨), activated by OKT3 (50 μg/ml; plastic-bound; (▨) or NKIL16 (final dilution 1 : 50 culture SN; ■). The percentages of control T-B conjugates were: for resting $CD4^+$ T cells: 12 ± 2%, for anti-CD3-preactivated $CD4^+$ T cells: 21 ± 2% and for NKIL16-preactivated $CD4^+$ T: 24 ± 3. The results of one typical experiment (mean ± SD of duplicates) among five experiments are shown.

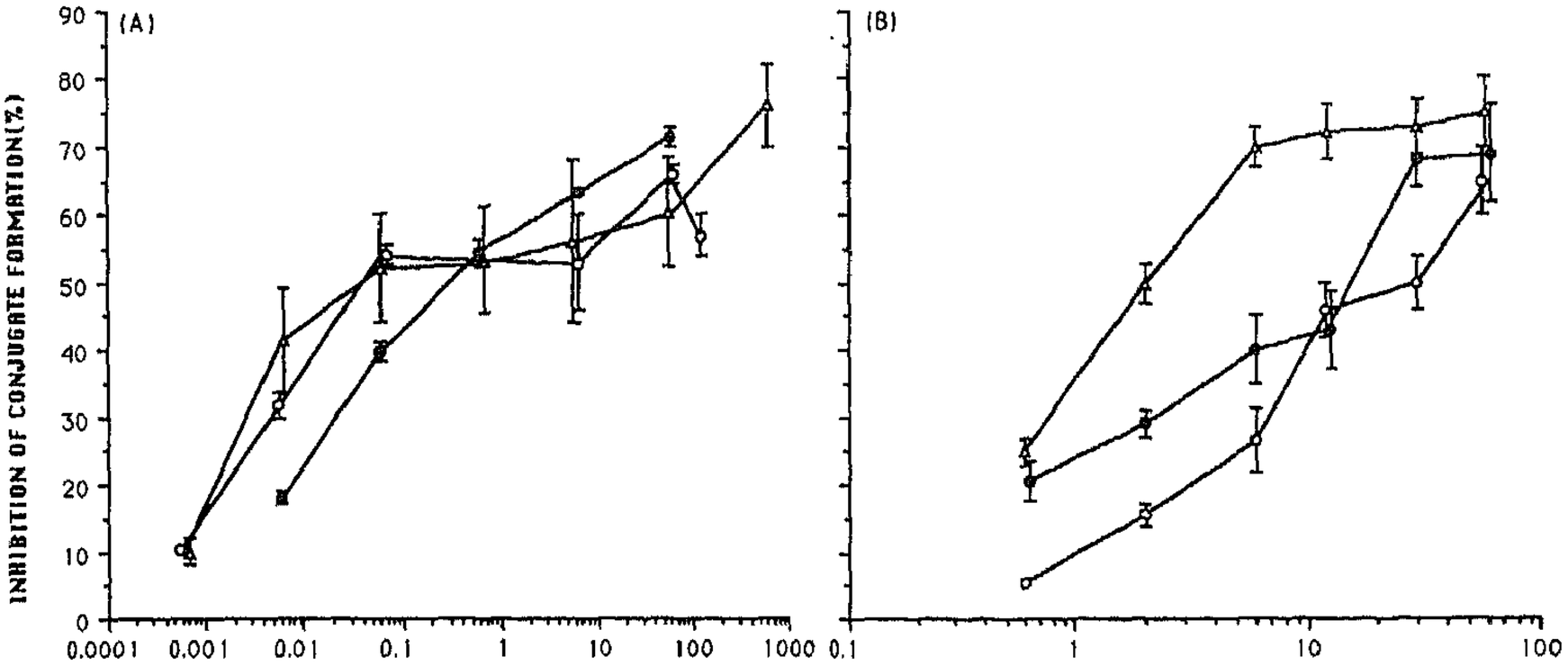

Figure 5. Inhibition of anti-CD3- and NKIL16-activated $CD4^+$ T cell adhesion by gp160 and by MHCS class II-derived peptides. gp160 (A), and DR-12 peptide (B) were incubated with resting (△), OKT3-preactivated (●), or NKIL16-preactivated (○) $CD4^+$ T cells during conjugate formation with HLA class II^+ B cells. The percentages of control T-B conjugates were: for resting T cells: 11 ± 1%, for OKT3-preactivated T cells: 21 ± 2%, for NKIL16-preactivated T cells: 26 ± 3%. Peptide DR43-54 and class I 31-42 had an inhibitory activity of < 1% on OKT3-treated T cell adhesion and of < 3% on NKIL16-treated T cell adhesion respectively. The results of one typical experiment (mean ± SD of duplicates) among six experiments are shown.

sion of $CD4^+$ T cells to MHC class II^- (Fig. 4 B) and MHC class II^+ B cells (Fig. 4 a). Purified gp160 similarly inhibited the adhesion of resting and anti-CD3- and NKIL16-activated $CD4^+$ T cells (Fig. 5 A). The DR-12 peptide analogous to position 35 to 46 of MHC class II β was significantly more active in inhibiting the adhesion of resting $CD4^+$ T cells than that of anti-CD3-activated T cells ($p < 0.0001$). The NK1L16-activated $CD4^+$ T cell adhesion was less sensitive to DR-12-mediated inhibition for DR-12 concentrations below 10 μM (Fig. 5 B; $p < 0.001$). Control 12-mer peptides (class I 31–42, DR 43–54) and DR12 variants (DR12 V1, DR12 V2, DR12 V4) had no effect. Further evidence of specificity of the inhibition by DR12 was also provided by preincubation of this peptide with soluble CD4 that led to neutralization of the inhibitory effect of DR12 (Fig. 6). It is noteworthy that neutralization by soluble CD4 of the DR12 peptide inhibitory effect was not equimolar, suggesting that not all peptides had the optimal conformation, as already observed in previous experiments [6]. Finally, two gp160-derived peptides analogous to positions 418–434 and 449–464, respectively, known to participate to the binding of gp160 to CD4 [18] were more potent in suppressing the adhesion of resting $CD4^+$ T cells than that of anti-CD3- or NK1L16-activated T cells (Fig. 7; $p < 0.001$ and $p < 0.0001$, respectively, for concentrations of 0.4 μM and 4 μM). However, identical maximal inhibition of adhesion could be reached with the two peptides. A control peptide (302–324) derived from gp160 had no effect.

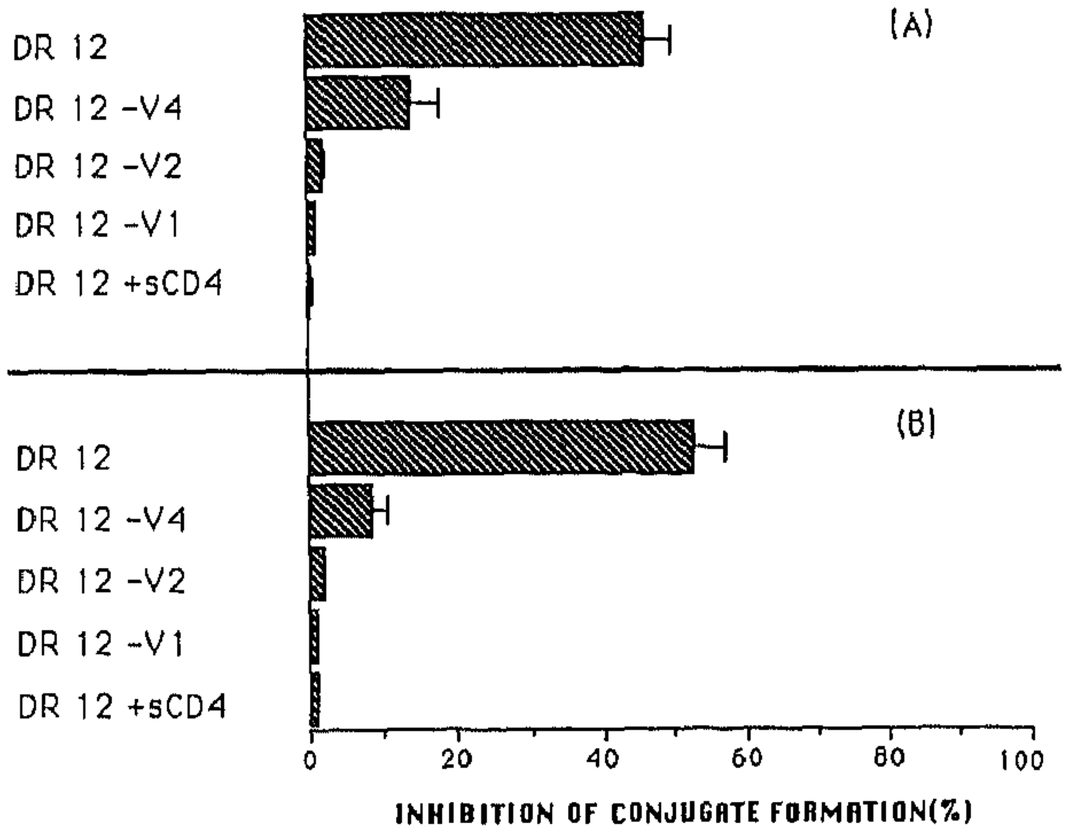

Figure 6. Specific inhibition of anti-CD3- and NKIL16-activated $CD4^+$ T cell adhesion by DR-12 (35-46). DR-12 (35-46) and DR-12 (35-46) variants peptides were incubated (at 12 μM with NKIL16-preactivated $CD4^+$) T cells in (A) or OKT3-DR-12 (35-46; 12 μM) was preincubated with soluble CD4 (at 200 nM) during 10 min at 37 °C before incubation with T and B cells for conjugate formation. The percentages of control T-B conjugastes were: (A): 23 ± 2% and (B): 19 ± 2%. The results of one typical experiment (mean ± SD of duplicates) among five experiments are shown.

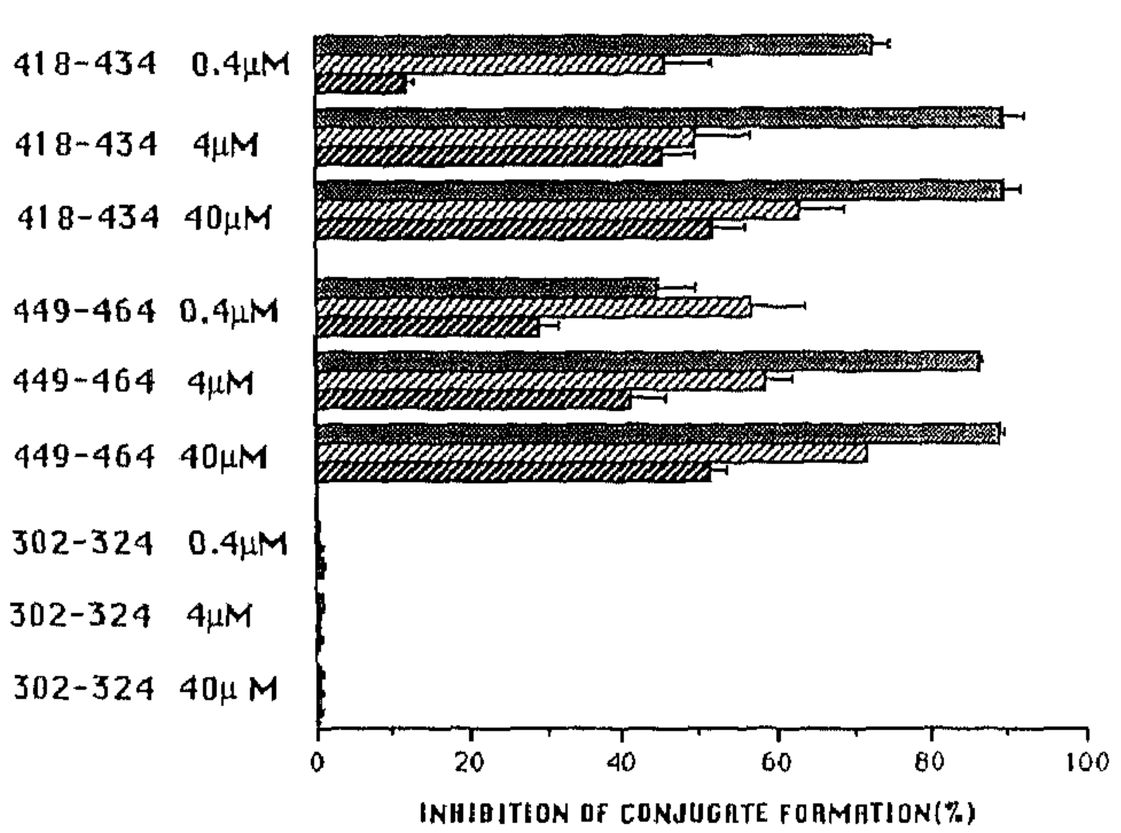

Figure 7. Inhibition of anti-CD3- and of NKIL16-activated $CD4^+$ T cells by gp160-derived peptides. Resting (▩) NKIL16-preactivated (▨) or OKT3-preactivated (▨) $CD4^+$ T cells were incubated during conjugate formation with HLA class II^+ B cells with gp160-derived peptides (418-434, 449-464, 302-324). The percentages of control T-B conjugates were: for resting T cells: 9 ± 2%, for NKIL16-preactivated T cells: 21 ± 2% and for OKT3-preactivated T cells: 21 ± 2%. The results of one typical experiment (mean ± SD of duplicates) among four experiments are shown.

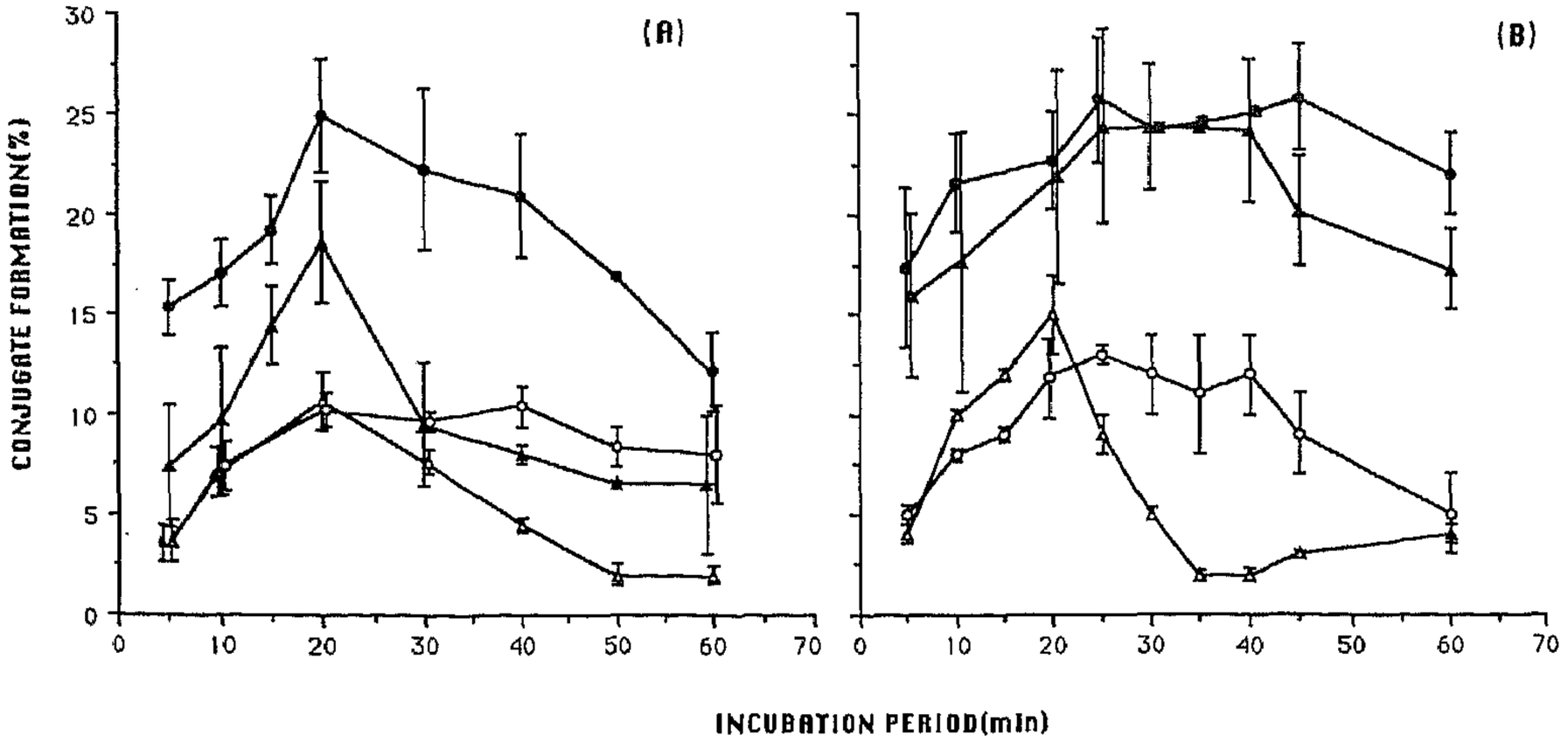

Figure 8. Kinetics of adhesion of anti-CD3- and NKI-L16 activated $CD4^+$ T cells to MHC class II^+ and MHC class II^- B cell lines. (A): HLA class II^+ B cells were incubated with resting (△) or OKT3-preactivated $CD4^+$ T cells (▲). HLA c lass II^- B cells (RJ225) were incubated with resting (○) or OKT3-preactivated $CD4^+$ T cells (●). (B): HLA class II^+ B cells were incubated with resting (△) or NKIL16-activated $CD4^+$ T cells (▲). HLA class II^- B cells were incubated with resting (○) or NKIL16-activated $CD4^+$ Tcells (●). Results are the mean of six experiments ± 1 SD. Similar results were obtained by using an EBV-B cell line from an immunodeficient patient with defective expression of HLA class II molecules ([24]; data not shown).

3.3 Kinetics of adhesion of anti-CD3-activated $CD4^+$ T cells to MHC class II^+ and MHC class II^- B cell lines

As we have previously shown [6], the kinetics of adhesion of resting $CD4^+$ Tcells to MHC class II^+ and MHC class II^- B cells differed strongly (Fig. 8): Adhesion to class II^- B cells is prolonged, remaining at a plateau for 20 to 40 min, whereas adhesion to class II^+ B cells peaks and then drops off rapidly. It has been suggested that this MHC class II-related alteration of adhesion reflects a negative regulation of T cell adhesion mediated by weak interactions between CD4 and MHC class II molecules [6]. We, therefore, tested whether such regulation was active in anti-CD3-activated $CD4^+$ Tcells. As shown in Fig. 8 a, we found almost identical maximal binding of anti-CD3-activated $CD4^+$ Tcells to MHC class II^+ and MHC class II^- B cells. This was shown using both mutant RJ225 B cells and B cells from patients with defective MHC class II expression; these B cells express similar levels of the adhesion molecules ICAM-1 and LFA-3 (data not shown). The kinetics of adhesion of anti-CD3-activated $CD4^+$ T cell to MHC class II^- B cells did not follow a plateau similar to that of resting $CD4^+$ T cells. The slope of the decrease in T-B conjugates between 20 and 60 min was, however, significantly shallower than that observed with MHC class II^+ B cells (Fig. 8 a; $p < 0.01$). NKIL16-activated T cells bound MHC class II^+ and MHC class II^- B cells to the same extent, with a moderate decrease in conjugates formation after 40 min (Fig. 8 b).

4 Discussion

In this work, we studied the adhesion of $CD4^+$ Tcells that had been activated either by CD3 cross-linking or by the CD11a-sepcific antibody NKIL16. We confirm that in both instances, the observed up-regulation of adhesion, compared to resting cells is LFA-1 dependent. In addition, CD2 was again found to be involved in the adhesion pathway. This up-regulated adhesion was inhibited by anti-CD4 antibodies and gp160 and was partially inhibited by gp160-derived peptides. The adhesion of anti-CD3 antibody-activated $CD4^+$ T cells was also partially inhibited by the DR-12 peptide which mimicks the 35–46 sequence of MHC class II β molecules whereas the adhesion of NKIL16-activated $CD4^+$ T cells was only slightly inhibited. In addition, anti-CD3 antibody- and NKIL16-activated $CD4^+$ Tcells bound equally to B cells regardless of MHC class II expression. The drop of adhesion of CD3-cross-linked $CD4^+$ T cells to MHC class II^+ B cells after a 20 min incubation was more pronounced than that observed with class II^- B cells.

These results can be interpreted in several ways. The CD4 molecule could be involved as an adhesion molecule in CD3- or LFA-1-preactivated $CD4^+$ T cell adhesion. This is supported by the significant role of CD4 in antigen-specific T cell adhesion [27, 28]. Similarly, it has been recently shown that in a long-term (1 to 2.5 h) adhesion assay TcR triggering could up-regulate the CD8-MHC class I interaction [29]. However, it has been found that CD4 has no detectable role in mediating antigen-independent adhesion of resting or clonal $CD4^+$ T cell populations [6, 26]. In the system we studied no difference in the adhesion of activated $CD4^+$ T cells to MHC class II^+ and MHC class II^- B cells was detected. This distinguishes these models from MHC class II-restricted binding of T cells to murine fibroblasts transfected with human MHC class II molecules [11], where a TcR-MHC class II molecule interaction mediates adhesion.

These results do not rule out the hypothesis that CD4 is involved in the up-regulation of adhesion induced by CD3 cross-linking, since CD4-CD3 association is known to occur in T cell activation [30, 31] and could facilitate signal transduction leading to up-regulation of adhesion [14, 32]. This question could be only addressed by testing inhibition of adhesion by the addition of CD4 ligands at the time of CD3 cross-linking or by studying the effect of CD3 cross-linking on $CD4^-CD3^+$ T cells.

CD4 ligands could inhibit up-regulation of adhesion by inducing dissociation of a CD3/TcR/CD4 complex. This explanation could account for the inhibition of CD3-mediated up-regulation of T cell adhesion but not that of LFA-1-mediated up-regulation. The latter is known not to depend upon a CD4-CD3/TcR interaction [15].

The present results are, thus, compatible with the hypothesis that CD4 ligands and putative CD4 ligands (gp160 and MHC class II-derived peptides) can inhibit through negative signaling of both activated and resting T cell adhesion ([6, 33, 34]; Mazerolles et al., *Hum. Immunol.*, in press). Indeed, having made similar observations in the study of the adhesion of resting $CD4^+$ T cells, we proposed that CD4 can transduce a signal negatively regulating cell adhesion after ligand binding ([6]; Corado et al., submitted and Mazerolles et al., *Hum. Immunol.*, in press). CD4-CD4 ligand interaction would exert negative effects on T cell adhesion and possibly T cell activation [35, 36] when the CD4 ligand does not cross-link CD4 and the TcR/CD3 complex; this would contrast with the antigenic peptide-MHC class II complex which induces a positive signal [30, 31]. A similar mechanism could also control the adhesion of CD3-cross-linked T cells and of LFA-1-stimulated T cells. CD4 cross-linking would not be required as proposed by Hague et al. [37], since gp160 was found to be effective.

However, putative CD4 ligands (gp160-derived peptides that mimick the gp160 binding site to CD4 [18] and the DR-12 peptide analogous to the 35–46 sequence of HLA class II β, including the highly preserved RFDS sequence) that specifically inhibit adhesion of resting $CD4^+$ T cells to B cells were less active on T cells preactivated by CD3 cross-linking. These results, differing from those observed with resting $CD4^+$ T cells, may be explained by the higher affinity of LFA-1-mediated interactions [17]. It is likely that peptides are less active on T cells that display a higher affinity for their targets. Indeed, Dustin and Springer demonstrated that the anti-CD3-induced up-regulation of adhesion relies on an increased affinity of LFA-1 for its ligand since LFA-1-dependent up-regulation of adhesion was not associated with a rise in the number of membrane LFA-1 molecules [14, 16]. They also demonstrated that this up-regulation was an active and transient phenomenon. In addition, the adhesion of such T cells to purified ICAM-1 was also transient. This indicates that negative regulation of this process results in part from cessation of the positive signaling induced by CD3 cross-linking which may be mediated by PKC activation and phosphorylation of either LFA-1, or of an associated molecule [14, 16]. This was also found in our experimental model since down-regulation of anti-CD3-activated T cell adhesion was also observed in the absence of CD4-MHC class II interaction by studying adhesion to MHC class II^- B cells. Here, we demonstrate that the CD3-dependent up-regulation of LFA-1-dependent adhesion can also be down-regulated by CD4-CD4 ligand interaction. Indeed CD3-activated $CD4^+$ T cells bind in a more prolonged manner to MHC class II^- B cells than to MHC class II^+ B cells; furthermore, CD4 ligands partially inhibited the CD3-mediated up-regulation of T cell adhesion and anti-CD4 antibodies inhibit the interaction of anti-CD3 antibody-activated $CD4^+$ T cells with MHC class II^- B cells. This effect is not secondary to a CD4-induced LFA-1 modulation since LFA-1 expression is not modified in the presence of CD4 ligands ([6]; Corado et al., submitted).

As previously demonstrated by Keizer et al. [15] for homotypic adhesion, the NKIL16-mediated up-regulation of T cell adhesion to B cells is relatively stable. No influence of potential CD4-MHC class II interaction on the kinetics of T-B cell adhesion was found. In addition, putative CD4 ligands had moderate inhibitory activities on the adhesion of NKIL16-treated $CD4^+$ T cells. The NKIL16 antibody is thought to increase the affinity of LFA-1 for its ligand, ICAM-1, by passively inducing a conformational change in the LFA-1 molecule [15]. It is, therefore, not surprising that the CD4-MHC class II interaction is far less involved in the regulation of such LFA-1-mediated T cell adhesion. The gp160 is in contrast similarly able to inhibit adhesion of resting, anti-CD3- and NKIL16-pretreated T cells, suggesting that its effect either is more potent or relies on a different mechanism. The former hypothesis is more likely since the affinity of gp160 for CD4 is much higher than that of MHC class II for CD4 [18].

Taken together, our results suggest that in addition to down-regulating low-affinity antigen-independent T cell adhesion, the CD4-MHC class II interaction may also partially down-regulate high-affinity T cell adhesion induced by CD3 cross-linking. It is tempting to speculate that *in vivo*, this molecular interaction may prevent antigen-independent T cell adhesion and may also contribute to terminating antigen-specific T cell adhesion up-regulated by CD3/TcR cross-linking. This should imply either CD4-CD3/TcR dissociation or modulation of the CD4-CD3/TcR complexes formed during T cell activation, sparing CD4 molecules not associated with the CD3/TcR complex and able to transduce a signal down-regulating T cell adhesion. The mechanism of this signal is at present unknown, but may involve the p56lck tyrosine kinase that is associated with CD4 [38].

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