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COOPERATIVE AGREEMENT PROGRAM NCC 9-36, ROUND II

COVER SHEET FOR FINAL REPORT

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Institution:	**UT M. D. Anderson Cancer Center** **Science Park, Department of Veterinary Sciences**
Name of Project:	**"Studies of Cell-Mediated Immunity Against Immune Disorders Using Synthetic Peptides and Rotating Bioreactor System"**
Amount of Grant:	[redacted]
* Amount Spent, if Different from Amount Granted:	**N/A**
Date of Project Completion:	**April 30, 1998**

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FINAL REPORT: APRIL 1998
NASA contract: NCC 9-36, Round II

We conducted a series of experiments using mouse immune-precursor cells, and observed that bioreactor culturing results in the loss of antigen-specific cytotoxic T lymphocyte (CTL) function. The reason for the abrogation of CTL function is microgravity conditions in the bioreactor, but not the antigen per se or its MHC restriction. Similarly, we observed that allostimulation of human PBMC in the bioreactor, but not in the T flask, resulted in the blunting of both allo-CTL function and the NK activity, indicating that the microgravity-associated functional defects are not unique to the mouse system. These results provide further confirmation to the microgravity-associated immune dysfunction, and constitute ground-based confirmatory data for those related to the space-travel.

The following is a detailed summary of the results of the entire project study:

We employed a novel mouse model developed in our laboratory that uses non-infectious synthetic peptides for in vivo induction of CTLs capable of killing cells infected with infectious viruses (17-23). The major goal was to test whether the rotating bioreactor technology will be superior to conventional tissue culturing for expansion of cytotoxic T lymphocyte (CTL) precursors. Towards this goal, we have immunized Balb/c mice (expressing the MHC class I haplotype H-2^d) with a synthetic peptide from the immunodominent V3-loop region of the envelope protein gp120 of the human immunodeficiency virus type 1 (HIV-1). This peptide has previously been shown in our laboratory to be capable of inducing efficient CTL response directed against syngeneic target cells expressing the HIV-1 envelope protein, in the mouse model (17-21). After a 7 d period subsequent to intradermal immunization in the hind foot pad, mice were sacrificed and the draining popliteal lymph nodes (PLN) were surgically removed. Cells obtained from the PLN were restimulated for 5 d in the presence of irradiated syngeneic mouse spleen cells that were preincubated with the HIV peptide for 2 h at 37 C. For the restimulation, equal numbers of cells were cultured in the slow-turning lateral vessel (STLV), the high-aspect rotating vessel (HARV) or, in the tissue culture flask (T flask). At the end of the 5 d restimulation, cells were washed, counted and tested for cytolytic activity against syngeneic p815 cells that were either preincubated with medium alone or in the presence of the HIV synthetic peptide. Data presented in Fig. 1 clearly demonstrates that while T flask cultures showed high levels of antigen-specific CTL activity, those cultured in either STLV or HARV showed complete abrogation of the CTL activity. Similar results showing abrogation of antigen-specific CTL activity in bioreactor cultures were obtained when the experiment was repeated with a CTL epitope peptide (H-2^d-restricted) from a different virus, lymphocytic choriomeningitis virus (LCMV). These data from experiments employing HIV and LCMV peptides strongly suggest that the abrogation of CTL function is not unique to the peptide antigen employed.

We then sought to verify whether the MHC haplotype expressed by the mouse strain is a contributing factor for the observed abrogation of CTL activity. We conducted experiments in C57BL/6 mice which express the H-2^b haplotype, using a peptide, E31, corresponding to the E6 oncoprotein of human papillomavirus type 16 (HPV-16), that we previously demonstrated to be

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capable of inducing CTL (22). Restimulation of CTL precursors from E31-immunized C57BL/6 mice in the bioreactors drastically reduced the peptide-specific CTL activity, while those cultured in the T flask showed significant activity (Fig. 2). To further confirm that neither the antigen (i.e. peptide) nor the host MHC (i.e. the H2 haplotype) are the potential contributors for abrogation of CTL function. We immunized a hybrid mouse strain, CB6F1 that expresses H-2^{bxd}, with a mixture of 5 synthetic peptides corresponding to immunogenic proteins from five different viruses (HIV-1, HPV-16, LCMV, Influenza, and Sendai virus). Once again, restimulation of immune lymph node cells by culturing in the T flask with the peptide mixture resulted in elaboration of CTL activity specific to each of the peptides, while cultures from the bioreactor were completely devoid of CTL activity specific to any of the five peptides (Fig. 3). These results from studies in multiple mouse strains employing a variety of antigenic peptides clearly establish that the microgravity culturing of immune precursor cells in the bioreactors as responsible for the abrogation of antigen-specific CTL activity.

In an attempt to begin understanding the basis for the abrogation of CTL function of the cultures subjected to growth in the bioreactors, we first analyzed for differences in cell viability between the two growth conditions (Table 1). Overall, bioreactor cultures showed significantly lower viable cell populations ($p = 0.05$). A comparison of FACS analysis data from three different experiments presented in Table 1 indicates that the loss of CTL function in bioreactor cultures is not associated with changes in the viability of the CD8+ effector cell populations. We also did not observe significant differences in the numbers of viable cells expressing DEC-205, the receptor on dendritic cells which are known to function as potent antigen presenting cells (APC). On the other hand, a significant reduction was observed in the CD4+ cells ($p = 0.05$). Further systematic comparative analysis of cellular phenotype and functional properties of various cell types is required for a better understanding of the basis of microgravity-associated immune dysfunction.

We also conducted studies using human PBMC to test whether the functional defects we observed in mouse immune cells resulting from bioreactor culturing are unique to the mouse system. Specifically, we analyzed for differences in NK cells, and alloreactive CTL after stimulating the cells in either T flask or the bioreactor for seven days. Results presented in Fig. 4 clearly indicate that both the NK and allo-CTL function are severely compromised by culturing in the bioreactor. These results provide further confirmation to the microgravity-associated immune dysfunction, and also constitute ground-based confirmatroy data for those observed in astronauts and cosmonauts after both short- and long-duration space travel.

More recently, we were successful in adopting an in vitro immunization protocol (see methods section for details) for generating antigen-specific T cell responses with human PBMC (Table 2). In these studies, we have cultured DC isolated from human peripheral blood samples in vitro in the presence of GM-CSF and IL-4, and pulsed with synthetic peptides from highly conserved regions in the envelope protein gp120 of HIV-1. Subsequently, these cells were co-cultured with autologous lymphocytes. The resulting activated lymphocytes exhibited predominantly T cell phenotype, and showed efficient proliferative responses specific to the cognate HIV peptides. Such an in vitro protocol efficient in priming primary immune response specific to given antigen will have direct clinical utility in terms of fortifying immune responses in cases where body's natural capacity to mount an effective immune response is compromised. We plan to test whether the bioreactor technology, by virtue of its capacity to create 3D and in vivo -like environment, provides any benefits to enhance any of the aspects of this in vitro immunization protocol.

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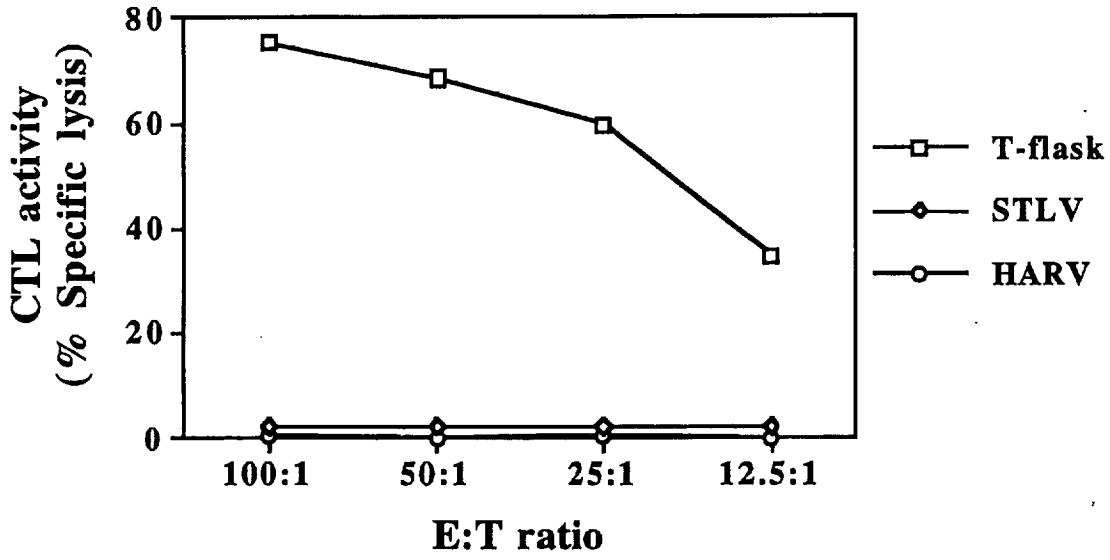

Fig. 1. Effect of microgravity culturing on the CTL activity of lymph node cells from Balb/c mice immunized with a synthetic peptide from the envelope protein of HIV-1

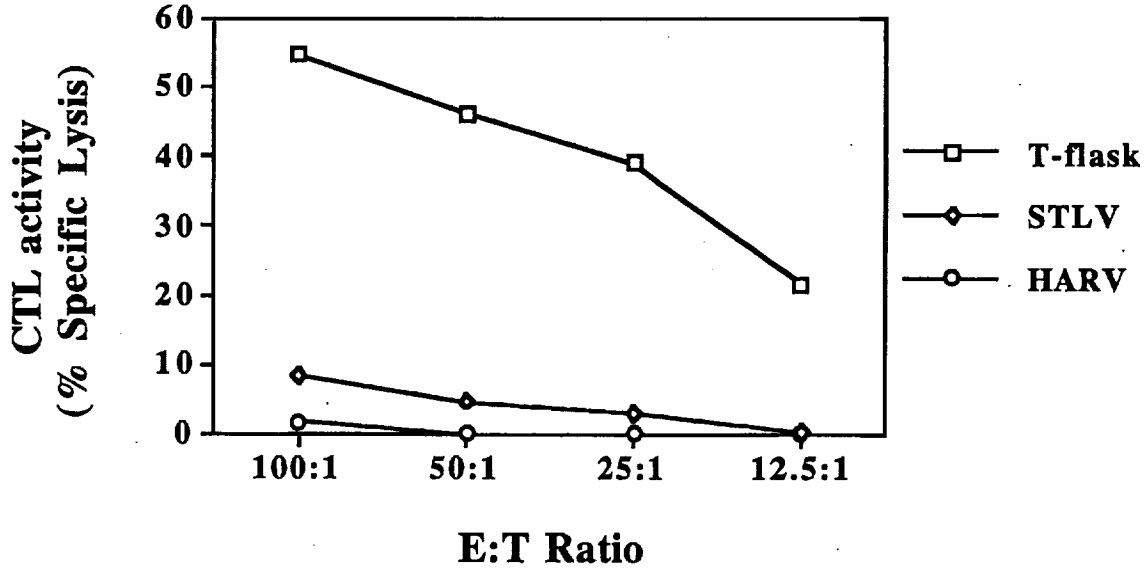

Fig. 2. Effect of microgravity culturing on the CTL activity of lymph node cells from C57Bl/6 mice immunized with a synthetic peptide from the E6 oncoprotein of HPV-16

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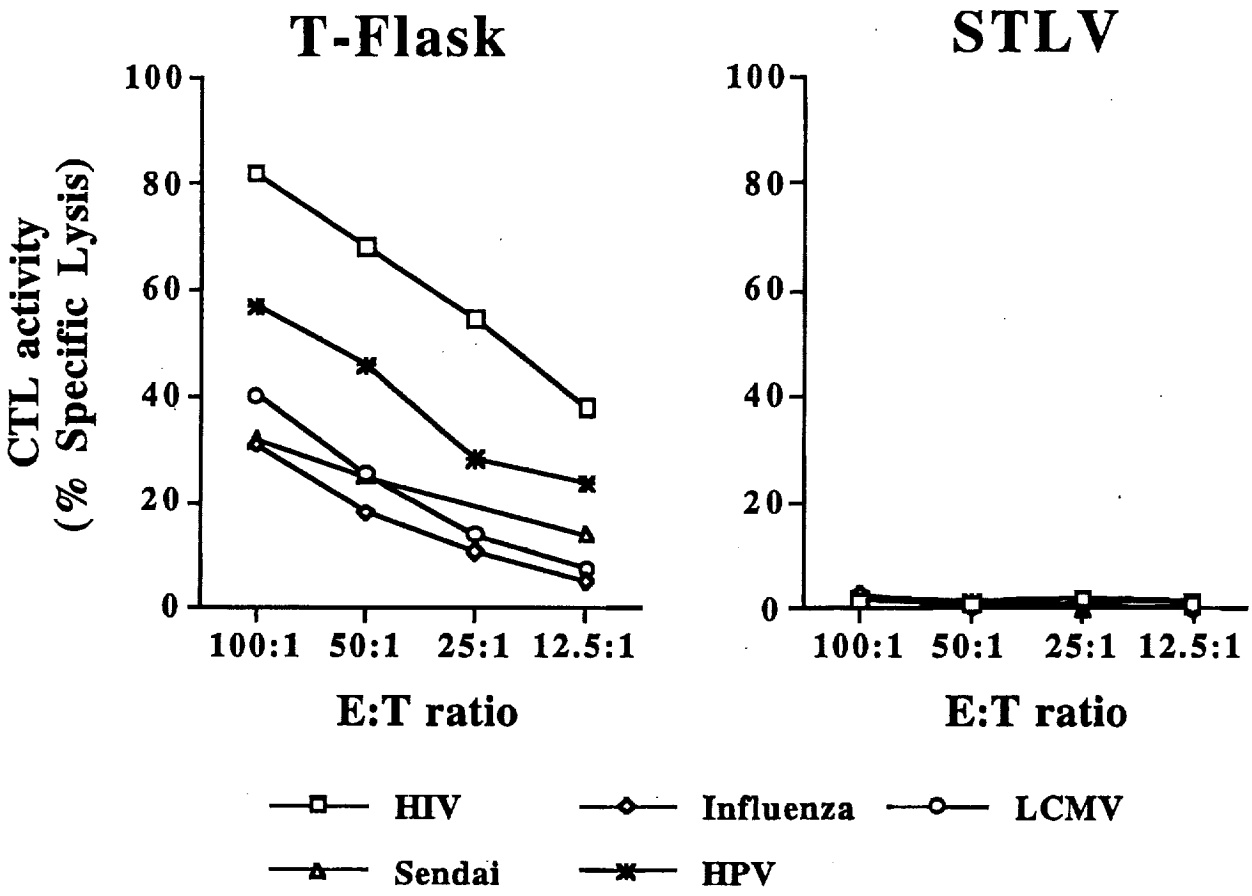

Fig. 3. Effect of microgravity culturing on the CTL activity of lymph node cells from CB6F1 hybrid mice immunized with a mixture of synthetic peptides from five viruses

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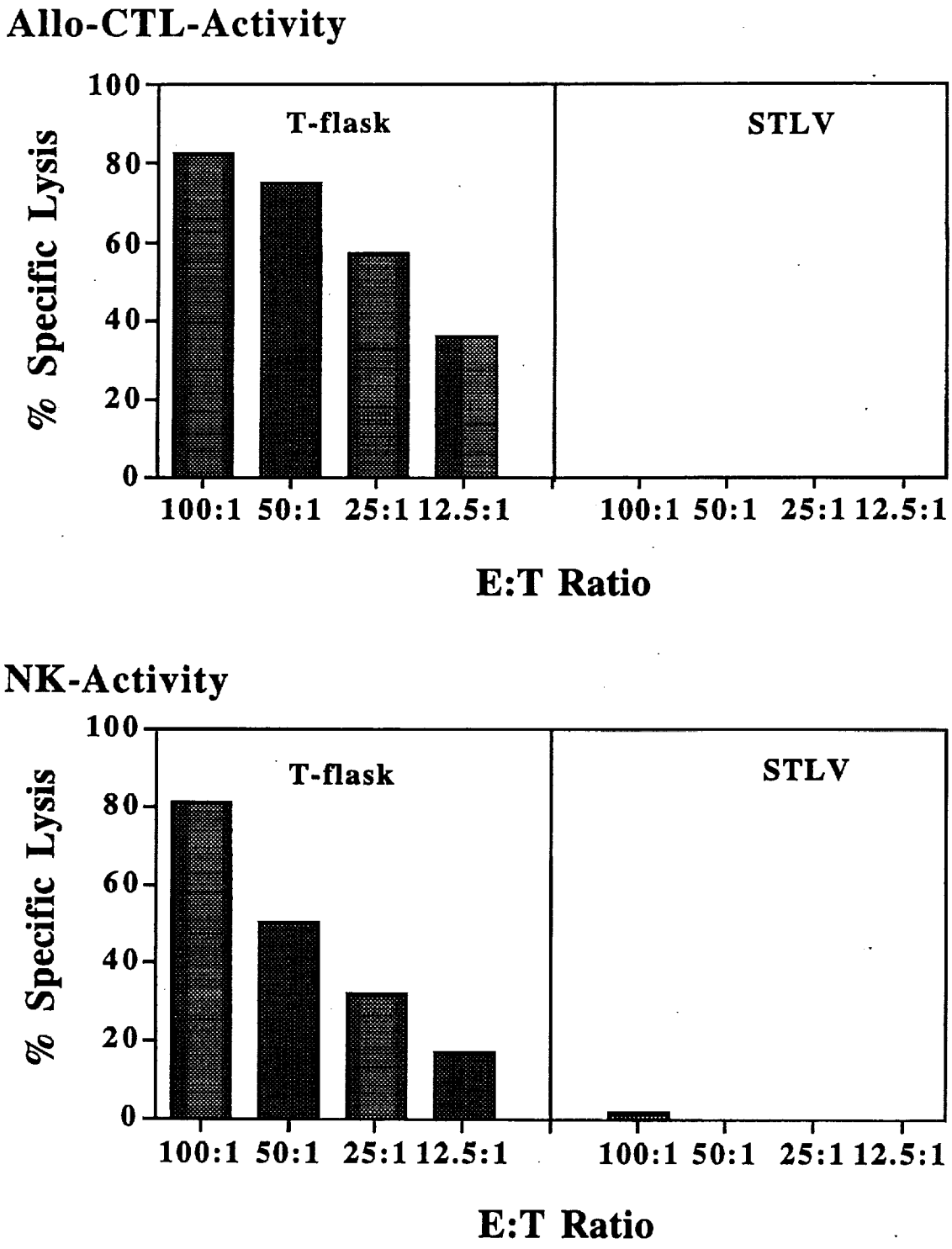

Fig. 4. Effect of microgravity culturing on the cytolytic function of human periferal blood mononuclear cells

Table: 1. Analysis of immune lymph node cells from Balb/c mice

Growth Condition	Total Cells Surviving (%)	CD4-Cells (%)	CD8-Cells (%)	Dendritic Cells (%)
2D-Cultures				
Experiment 1	27.9	32.1	48.5	26.2
Experiment 2	53.7	39.7	10.1	49.1
Experiment 3	43.6	48.4	23.9	15.7
Average	41.73 ± 13*	40.07 ± 8.2	27.5 ± 19.5	30.33 ± 17.1
3D-Cultures				
Experiment 1	18.2	24.3	45.5	15.4
Experiment 2	20.5	31.9	8.9	37.8
Experiment 3	25.0	28.9	5.3	8.9
Average	21.23 ± 3.5	28.37 ± 3.8	19.9 ± 22.2	20.7 ± 15.2
p ** =	0.05	0.05	0.28	0.28

* Standard Error; ** Mann-Whitney U

Table: 2. Proliferative responses of T cells primed by in vitro immunization with HIV peptides

Treatment	Proliferative Response (SI)
Medium	1.0
PHA	5.6
PPD	5.4
HIV peptides	10.2

Based on our pilot data, we hypothesize that the NASA bioreactor technology offers an efficient means to mimic and explore the mechanistic basis for the apparent immunodeficiency related to space-travel. It is important to determine the correlates to defects in effector T cell function resulting from RWV-culturing of immune effector cells from mice and humans. Comparative analysis should be conducted to determine: (i) the qualitative and quantitative differences in populations of various effector T cells (TH and CTL) and accessory cells (APC like dendritic cells), (ii) the accessory cell functions (antigen presentation by DC, and proliferation and cytokine production by TH cells), and (iii) the signal transduction mechanism involving the JAK-STAT molecules. Similar studies can be conducted with the human samples, but focusing initially on comparing allo-CTL and NK activities of cells cultured in the T flask and bioreactor. Subsequently, these two culturing protocols can also be compared for in vitro priming of synthetic peptide-specific T cell responses. For these experiments, DC isolated from human blood samples can be pulsed with synthetic peptides from HIV and HPV before co-culturing with autologous T cells either in T flask or bioreactor.

Since there are interesting parallels to microgravity-associated immune dysfunction in immune disorders like HIV-AIDS, and HPV-associated cervical cancer, we believe that the RWV technology for ex vivo manipulation of immune effector cells, yet mimicking immunological abnormalities observed in vivo, can provide valuable basic information about immune dysfunction related to these respective virus. Future studies in microgravity research should help expand the applicability of the NASA-bioreactor technology beyond the space science, to medicine, for developing strategies to efficiently prime, supplement, and reconstitute CMI responses against these immune disorders.