Modulation of human Vα24⁺Vβ11⁺ NKT cells by age, malignancy and conventional anticancer therapies

T Crough¹,², DM Purdie², M Okai², A Maksoud², M Nieda³ and AJ Nicol*¹,¹,²,⁴

¹Department of Medicine, University of Queensland, Brisbane, Australia; ²The Queensland Institute of Medical Research, Brisbane, Australia; ³Bone Marrow Transplant Unit, Royal Brisbane Hospital, Brisbane, Australia; ⁴Yokohama City University School of Medicine, Japan

Immunotherapy strategies aimed at increasing human Vα24⁺Vβ11⁺ natural killer T (NKT) cell numbers are currently a major focus. To provide further information towards the goal of NKT cell-based immunotherapy, we assessed the effects of age, cancer status and prior anticancer treatment on NKT cell numbers and their expansion capacity following α-galactosylceramide (α-GalCer) stimulation. The percentage and absolute number of peripheral blood NKT cells was assessed in 40 healthy donors and 109 solid cancer patients (colorectal (n = 33), breast (n = 10), melanoma (n = 17), lung (n = 8), renal cell carcinoma (n = 10), other cancers (n = 31)). Responsiveness to α-GalCer stimulation was also assessed in 28 of the cancer patients and 37 of the healthy donors. Natural killer T cell numbers were significantly reduced in melanoma and breast cancer patients. While NKT numbers decreased with age in healthy donors, NKT cells were decreased in these cancer subgroups despite age and sex adjustments. Prior radiation treatment was shown to contribute to the observed reduction in melanoma patients. Although cancer patient NKT cells were significantly less responsive to α-GalCer stimulation, they remained capable of substantial expansion. Natural killer T cells are therefore modulated by age, malignancy and prior anticancer treatment; however, cancer patient NKT cells remain capable of responding to α-GalCer-based immunotherapies.

British Journal of Cancer (2004) 91, 1880–1886. doi:10.1038/sj.bjc.6602218 www.bjcancer.com
Published online 2 November 2004
© 2004 Cancer Research UK

Keywords: NKT cells; immune therapy; age; human

Natural killer T (NKT) cells are a unique lymphocyte subset, which in humans are characterized by the presence of an invariant T-cell receptor (TCR) Vα24⁺Vβ11⁺ associated with the natural killer (NK) receptor NKR-P1A (Dellabona et al, 1994). Unlike conventional T cells human NKT cells and their murine counterpart, Vα14 NKT cells are activated by the glycolipid, α-galactosylceramide (α-GalCer) presented by a nonpoly- morphic major histocompatibility complex (MHC) class I-like molecule CD1d (Kawano et al, 1999b; Nieda et al, 1999). Natural killer T cells have been shown to have strong antitumour activity upon α-GalCer stimulation (Kawano et al, 1998; Nicol et al, 2000b; Kikuchi et al, 2001; Nieda et al, 2001). As a consequence, clinical trials aimed at activating and increasing NKT cell numbers are currently a major focus for immune therapy of cancer.

There are limited studies detailing the normal range of human peripheral blood (PB) NKT cells numbers and the effects of parameters such as age, malignant disease and associated treatment on their number and function. As the ageing process and immune dysfunction due to malignant disease have been shown to impact other cell types, these factors require consideration for NKT cells (Goto et al, 1999; Ninomiya et al, 1999; Hakim et al, 2004; Plackett et al, 2004).

The effect of the ageing process on NKT cell numbers has only been assessed in a single study, where the percentage of Vα24⁺ T cells was shown to decrease with age (DelaRosa et al, 2002). The impact of age, however, on NKT cell expansion following α-GalCer stimulation has not yet been evaluated (DelaRosa et al, 2002). If NKT cells do contribute to the normal protective mechanisms against cancer, a reduction in NKT cell number or function as a result of the ageing process age may contribute to the higher incidence of cancer in this group of patients.

The effects of malignancy on NKT cell number and function remain unclear. Significant reductions in PB NKT cell numbers have been reported in patients with melanoma, prostate cancer and lung cancer (Kawano et al, 1999a; Tahir et al, 2001; Motohashi et al, 2002), however in other studies the numbers of NKT cells in advanced cancer patients are comparable to those in healthy individuals (Yanagisawa et al, 2002). Variations in NKT cell function have also been described. Decreased responsiveness of NKT cells to α-galactosylceramide (α-GalCer) stimulation has been demonstrated in prostate cancer patients and advanced cancer patients (Tahir et al, 2001); however, in contrast NKT cells of lung cancer and melanoma patients have been shown to have comparatively normal α-GalCer proliferative capacity (Kawano et al, 1999a; Motohashi et al, 2002).
Natural killer T cells were defined as V\(\alpha\) were purchased from Beckman Coulter (Sydney, Australia). Appropriate isotype controls were used and all antibodies using a FACSCalibur (Becton Dickinson, San Diego CA, USA). Appropriate isotype controls were used and all antibodies were analysed by three-colour flow cytometry (IgG1, UCHT1) and phycoerythrin – cyanin 5.1 (PC5)-conjugated anti-CD3 (IgG1: Cl5), phycoerythrin (PE)-conjugated anti-V\(\beta\) and 28 of the cancer patients and 37 of the healthy donors following assessment of NKT cell numbers.

**MATERIALS AND METHODS**

**Subject groups and controls**

The study group consisted of 109 subjects (64 males and 45 females) with solid organ cancer (median age 58 years, range 26 – 82). They were subclassified according to cancer type (colorectal, breast, melanoma, renal cell carcinoma (RCC), lung, others) and prior cancer treatment. The control healthy volunteer group comprised 40 healthy volunteers, 25 females and 15 males (median age 34 years, range 19 – 68) with no history of malignant disease. Natural killer T cell number was assessed in each of the cancer patients and healthy donor, however only 37 of the healthy donors and 28 of the cancer patients could be assessed for expansion capacity following \(\gamma\)-GalCer stimulation.

Characteristics of the study group are presented in Table 1. Peripheral blood samples were collected after obtaining informed consent from the subjects, and the study was approved by The Royal Brisbane and Womens Hospital and the Health Services Ethics Committee.

**Phenotypic analysis**

Fresh whole blood or peripheral blood mononuclear cells (PBMC), separated by Ficoll – Paque (Amersham Pharmacia Biotech, Uppsala, Sweden) density centrifugation were stained with fluorescein isothiocyanate (FITC)-conjugated anti-V\(\gamma\)24 (IgG1: C15), phycoerythrin (PE)-conjugated anti-V\(\beta\)11 (IgG2a:C21) and phycoerythrin-cyanin 5.1 (PC5)-conjugated anti-CD3 (IgG1, UCHT1) and analysed by three-colour flow cytometry using a FACSCalibur (Becton Dickinson, San Diego CA, USA). Appropriate isotype controls were used and all antibodies were purchased from Beckman Coulter (Sydney, Australia). Natural killer T cells were defined as V\(\gamma\)24\(^+\)V\(\beta\)11\(^+\)CD3\(^+\) and to ensure accuracy of the flow cytometric evaluation up to 1 x 10\(^6\) cells were assessed in order to acquire >100 NKT cell events.

**Calculation of NKT cell numbers**

Natural killer T cell numbers were expressed as a percentage of the CD3\(^+\) lymphocyte population as determined by flow cytometry. To determined the number of NKT cells per litre (l) of blood, the fraction of lymphocytes that were NKT cells according to flow cytometry was multiplied by the number of lymphocytes per litre as determined by an automated full blood count on the same sample.

**Expansion of V\(\gamma\)24\(^+\)V\(\beta\)11\(^+\) NKT cells**

Peripheral blood mononuclear cells were assessed for NKT cell numbers as described above (Day 0) and cultured at 1 x 10\(^6\) cells ml\(^-1\) in AIM-V medium (Gibco BRL, Melbourne, Australia) supplemented with 10% normal human AB serum, 10Uml\(^-1\) interleukin-2 (IL-2) (R&D Systems, Sydney, Australia) and 100 ngml\(^-1\) KRN 7000 (a gift from Pharmaceutical Division, Kirin Brewery, Gunma, Japan) for 7 days. On Day 7, the cells were harvested, counted and analysed by flow cytometry as described above and the results are expressed in terms of fold expansion, as determined from the respective NKT numbers at Day 0 and following 7 days expansion.

**Statistics**

Results for NKT cell percentages and absolute numbers of NKT cells and CD3\(^+\) T cells have been presented as medians separately for the different cancer groups. Owing to the large number of zero NKT cell percentage counts, the distribution was extremely skewed to the right and thus standard normal distribution-based statistics were not appropriate. For crude analysis, Kruskal – Wallis nonparametric analysis of variance was used to compare median NKT cell percentages and NKT1\(^+\) between cancer groups. Two-way comparisons between the various cancer groups and healthy volunteers were performed using Mann–Whitney \(U\)-tests. Both NKT cell percentage and absolute NKT cell numbers followed an approximately negative-binomial distribution, and thus negative-binomial regression was used to evaluate the statistical significance of its association with tumour status, after adjusting for age and sex differences. The degree of association between age and NKT cell percentage or NKT1\(^+\), age and fold expansion and NKT percentage and fold expansion were calculated using a Spearman’s nonparametric correlation coefficient.

| Subject group     | n  | Age range | Median age | Sex     | Treatment history |
|-------------------|----|-----------|------------|---------|-------------------|
| Colorectal        | 33 | 43 – 79   | 61         | 22      | 13                |
| Breast            | 10 | 41 – 69   | 54         | 0       | 1                 |
| Other cancers     | 31 | 27 – 72   | 58         | 13      | 13                |
| Melanoma          | 17 | 26 – 83   | 59         | 14      | 13                |
| Lung cancer       | 8  | 33 – 72   | 55         | 6       | 13                |
| RCC               | 10 | 36 – 67   | 53         | 9       | 2                 |
| All cancer cases  | 55 | 26 – 83   | 58         | 64      | 50                |
| Healthy donors    | 40 | 19 – 68   | 34         | 15      | 40                |

No prior treatment Prior chemotherapy Prior radiation Prior chemotherapy and Radiotherapy

| No prior treatment | Prior chemotherapy | Prior radiation | Prior chemotherapy and Radiation |
|-------------------|--------------------|-----------------|----------------------------------|
| 13                | 18                 | 0               | 3                                |
| 1                 | 2                  | 0               | 7                                |
| 13                | 16                 | 1               | 2                                |
| 13                | 3                  | 3               | 1                                |
| 2                 | 2                  | 2               | 2                                |
| 9                 | 0                  | 1               | 0                                |
| 50                | 39                 | 7               | 15                               |
| 40                | NA                 | NA              | NA                               |

NA = not applicable.

© 2004 Cancer Research UK British Journal of Cancer (2004) 91(11), 1880 – 1886
RESULTS

Effect of malignancy on PB NKT cell numbers

Natural killer T cells as a percentage of CD3\(^{+}\) T cells (NKT\%) and the absolute number of NKT cells\(^{1–1}\) of PB were assessed in healthy donors\((n = 40)\) and cancer patients\((n = 109)\) with a broad range of malignancies. Natural killer T cells comprised up to 1.27\% of CD3\(^{+}\) T cells\(\text{(median value } 0.113\%\text{)}\) and 9.89 \times 10\(^{6}\)\(^{1–1}\) of PB in healthy donors, and up to 1.19\% of CD3\(^{+}\) T cells\(\text{(median value } 0.02\%\text{)}\) and 6.52 \times 10\(^{6}\)\(^{1–1}\) \text{(median value } 0.2 \times 10^{6}\text{)}\) of PB in all cancer patients combined\(\text{(Table 2)}\). No significant difference was observed between the frequency\((P = 0.28)\) or number\((P = 0.06)\) of circulating NKT cells in cancer patients and those in healthy donors after adjusting for age and sex differences. Analysis of the individual cancer subgroups showed melanoma patients\((n = 17)\) to have a significantly lower NKT\%\(\text{(median } 0.01\%, \text{ } P = 0.04)\) and NKT\(^{1–1}\)\(\text{(median } 0.17 \times 10^{6}\text{)}\),\(P = 0.01)\), and breast cancer patients\((n = 10)\) to have a reduced frequency of NKT cells\(\text{(median } 0.01, \text{ } P = 0.02)\) but comparatively similar absolute numbers of NKT cells\(\text{(}P = 0.13)\). Total CD3\(^{+}\) T cells\(^{1–1}\) of PB were significantly lower in all cancer groups with the exception of colorectal cancer and RCC, indicating that the reduced NKT\% observed in melanoma and breast cancer patients could not be attributed to increased numbers of total PB T cells.

Impact of prior radiation and/or chemotherapy treatment on NKT cell numbers

Statistical comparison was conducted between the NKT cell numbers of all cancer patients who had not undergone previous chemotherapy or radiation treatment and those who had not undergone each treatment respectively. No significant difference was observed. Furthermore, while the significant difference detected between healthy donors and lung cancer patients with no history of chemotherapy treatment\(\text{(}P = 0.01)\) may suggest chemotherapymediated modulation of NKT cell numbers; the low subject numbers\((n = 4)\) in this subgroup does not facilitate accurate analysis.

Effect of the ageing process on NKT cell numbers

The percentage and absolute number of circulating NKT cells in healthy donors weakly but significantly correlated with age such that NKT\% and NKT\(^{1–1}\) decreased with increasing age (Figure 1, Table 2). The percentage and absolute number of NKT cell percentages and absolute numbers of NKT cells and CD3\(^{+}\) T cells in cancer patients combined (Table 2) are shown.

Table 2  NKT cell percentages, absolute number of NKT cells\(^{1–1}\) \(\times 10^{6}\) and absolute number of CD3\(^{+}\) T cells\(^{1–1}\) \(\times 10^{6}\) of PB for each study group in comparison to healthy volunteers

| Subject group | n  | Range | Median | P-value | NKT % | Range | Median | P-value | NKT \(^{1–1}\) \(\times 10^{6}\) | Range | Median | P-value | CD3\(^{+}\) T cells\(^{1–1}\) \(\times 10^{6}\) | Range | Median | P-value |
|----------------|-----|-------|--------|---------|--------|-------|--------|---------|-------------------|-------|--------|---------|-------------------|-------|--------|---------|
| Colorectal     | 33  | 0–0.29| 0.012  | 0.17    | 0.00–6.53 | 0.17 | 0.15 | 0.019–1.87 | 1.08 | 0.06 |
| Breast         | 10  | 0–0.15| 0.01   | 0.02    | 0.00–3.32 | 0.12 | 0.13 | 0.25–2.10 | 1.00 | 0.01 |
| Other cancers* | 31  | 0–1.19| 0.04   | 0.86    | 0.00–3.24 | 0.31 | 0.20 | 0.16–2.24 | 0.90 | 0.003 |
| Melanoma       | 17  | 0–0.17| 0.01   | 0.04    | 0.00–1.61 | 0.17 | 0.01 | 0.25–2.04 | 0.69 | 0.004 |
| Lung cancer    | 8   | 0–0.26| 0.03   | 0.61    | 0.00–2.10 | 0.25 | 0.09 | 0.33–1.41 | 0.85 | 0.01 |
| RCC            | 10  | 0–0.77| 0.04   | 0.66    | 0.00–1.60 | 0.52 | 0.82 | 0.49–1.74 | 1.04 | 0.25 |
| All cancer cases | 109 | 0–1.19| 0.02   | 0.28    | 0.00–6.53 | 0.20 | 0.06 | 0.16–2.24 | 0.92 | <0.001 |
| Healthy Donors | 40  | 0–1.27| 0.11   | Reference | 0.00–9.89 | 1.93 | NA   | 0.66–4.52 | 1.43 | Reference |

NKT cell percentage is calculated as a percentage of the total CD3\(^{+}\) T cells as determined by flow cytometry. *P-values compared with healthy donors obtained from negative binomial regression models adjusted for age and sex differences between study groups. *Other cancers include liver, adenocarcinoma, prostate, ovarian, uterine and stomach cancer. NA = not applicable.

Table 3  Effects of prior radiation and chemotherapy treatment on NKT cell percentages and absolute numbers of NKT cells and CD3\(^{+}\) T cells in cancer patients

| Subject group             | n   | Median | P-value | NKT % | Range | Median | P-value | CD3\(^{+}\) T cells\(^{1–1}\) \(\times 10^{6}\) | Range | Median | P-value |
|---------------------------|-----|--------|---------|--------|-------|--------|---------|-------------------|-------|--------|---------|
| Prior radiation treatment | 22  | 0.02   | 0.95    | 0.14   | 0.28 | 0.82 | 0.10 |
| No prior radiation treatment | 87 | 0.02   | 0.24   | 1.00 |
| Prior chemotherapy treatment | 54 | 0.02   | 0.26 | 0.21 | 0.26 | 1.04 | 0.83 |
| No prior chemotherapy treatment | 55 | 0.03   | 0.87 |

*P-value obtained from negative binomial models adjusting for age and sex differences between the groups.
panels A and B). While the number of NKT as a percentage and per litre of blood of cancer patients also appeared to decrease with increasing age, this correlation proved insignificant (Figure 1, panels A and B).

Expansion capability of NKT cells in cancer patients and healthy volunteers – the effect of age and malignancy

Stimulation by α-GalCer resulted in the rapid expansion of NKT cells from most cancer patients and healthy donors. Figure 2 displays the expansion capacity (fold expansion) of healthy donor (n = 37) and cancer patient (n = 28) NKT cells in response to in vitro α-GalCer stimulation. Natural killer T cells of healthy donors expanded between 0- and 303-fold and those of cancer patients expanded between 0- and 959-fold. While NKT cells of cancer patients had an overall reduced capacity to expand (median eight-fold) as compared to healthy donors (median 43-fold, P = 0.02), some individual cancer patients were clearly better NKT cell expanders than healthy donors.

Contrary to the effects of age on NKT numbers in healthy donors, age did not significantly influence their fold expansion as displayed in Figure 1C. Although the proliferative potential of cancer patient NKT cells to α-GalCer appears to decrease significantly with age, conclusive analysis requires the assessment of increased numbers of younger cancer patients.

Although NKT cells expanded rapidly from most donors, there was no relationship between the NKT % in healthy donors and the degree to which the NKT cells expand. The results do suggest, however, that NKT % is a reliable indicator of fold expansion in cancer patients, although the highly skewed nature of the population towards low NKT % may make the spread of NKT % too low to determine the correlation accurately. It is of interest that the largest in vitro NKT cell expansion was observed from donors with relatively low NKT cell numbers.

**DISCUSSION**

Clinical application of NKT cells as therapy for malignancy is critically dependent upon their activation and subsequent expansion in humans. With the development of therapeutic approaches with the potential to activate NKT cells in vivo (Nieda et al, 2003) and expand NKT cells in vitro (Nieda et al, 1999; Nicol et al, 2000a; Takahashi et al, 2000), it is important to more clearly define their applicability in the clinical setting. Previous data indicate that NKT cells may be decreased in some cancer patients (Kawano et al, 1999a; Tahir et al, 2001; Motohashi et al, 2002). If this is associated with or caused by decreased responsiveness to α-GalCer, then these therapies may have less than expected clinical utilities.

We have confirmed with considerably larger subject numbers in this study that the percentage of Vα24+ NKT cells in cancer patients does not significantly differ to that in healthy donors when all cancer types are considered collectively (Yanagisawa et al, 2002). Our data also support previous reports that NKT cell numbers are significantly reduced in patients with melanoma (Kawano et al, 1999a); however, in contrast to Motohashi et al (2002) decreased frequency and absolute number of NKT cells was not seen in lung cancer patients. A likely explanation for this inconsistency is the inclusion of insufficient patients in this subgroup to detect a statistically significant difference, in view of the 60 lung cancer patients assessed in the previous study.

As no studies to date have assessed the numbers of NKT cells in breast cancer patients we have, for the first time, demonstrated a significantly reduced percentage of NKT cells in this cancer group.

Reasons for the observed reductions in breast cancer and melanoma patients are unknown. It is evident that the results are not simply a consequence of increased total CD3+ T cell numbers in either disease (Table 2). Contrary to previous studies, which have in majority assessed NKT cell levels pretreatment or without...
Human NKT cells, age and malignancy

Figure 1. Association of age on proportion of NKT cells (NKT %; A), absolute numbers of circulating NKT cells (NKT$^{-1}$; B) and responsiveness to α-GalCer (C) in cancer patients and healthy donors. (D) represents the correlation between NKT cell numbers and NKT cell expansion capacity.
of Vα24+ NKT cells in healthy elderly individuals. We have also demonstrated that absolute NKT cell numbers decrease significantly with age in healthy donors. A similar trend was observed in cancer patients, however the statistical significance (P = 0.06) was potentially influenced by a concentration of middle- to late-aged cancer patients. Although greater numbers of younger cancer patients are required to assess this association accurately, the results do raise the possibility that low NKT cells may be a risk factor for cancer in young adults. They also confirm that in consideration of their intrinsically low NKT cell numbers, middle and older aged cancer patients could benefit greatly from therapeutic manipulations that boost their NKT cell numbers.

The magnitude of NKT cell activation and expansion may have critical consequences on the efficacy of NKT cell therapies. We and others have shown that NKT cells of cancer patients can be dramatically expanded by α-GalCer stimulation, however to levels lower that that of healthy donors (Tahir et al, 2001). Studies of melanoma and lung cancer patients have in contrast reported NKT cells with comparatively normal capacities to expand (Kawano et al, 1999a; Motohashi et al, 2002). As we could not assess in vitro NKT expansion on every cancer subject involved, we cannot determine whether NKT cell expansion is reduced in every cancer type, or whether the overall result is influenced by one or several subgroups of cancer patients.

Mechanisms of reduced responsiveness of NKT cells to α-GalCer stimulation have not been determined. Immune defects and dysfunction in T cells and the antigen presenting and stimulatory capacity of DC have previously been reported in cancer patients (Mizoguchi et al, 1992; Goto et al, 1999; Kiessling et al, 1999). As in vitro expansion capacity is dependent upon both the number and stimulatory capacity of CD1d antigen-presenting cells, reduced numbers of and/or functionally defective CD1d APC may suppress NKT activation in cancer patients. Production of immunosuppressive factors by tumour cells or TGF-β by CD3+ T cells or intrinsic defects to the NKT cells themselves may also inhibit their expansion potential (Tada et al, 1991; Chouaib et al, 1997).

While functional impairment as a consequence of age has been reported to occur in other cell types such as T cells (Hakim et al, 2004; Plackett et al, 2004), there appears no association between the functional capacity of an NKT cell to respond to α-GalCer stimulation and the age of the healthy donor. Although fold expansion significantly decreased with age in cancer patients, this result was dependent upon three individuals with particularly high expansion. Confirmation of this latter observation would require the inclusion of an increased numbers of younger cancer patients in the subject group.

The precise relationship, if any, between the proportion of NKT cells in a donor and the NKT cell’s functional capacity to expand has to date not been examined. While we have shown that there is no association between the percentage of NKT cells and fold expansion in normal donors, it is clearly evident that the largest degree of expansion was in donors with an NKT cell % at the lower end of the scale. Although fold expansion significantly increased with NKT % in cancer patients, the highly skewed nature of the population towards low NKT % makes the spread of this variable too narrow to accurately determine the correlation. NKT cells capable of dramatic expansion, some indicating prior treatment, we have taken into consideration potential effects of chemotherapy or radiation treatment on NKT cell numbers. We have identified that radiation treatment may be directly responsible for the decreased NKT cell numbers in melanoma patients. It remains possible, however, that a disease-specific effect by melanoma may still contribute to the observed reduction and although not examined in this study, decreases in NKT cell numbers could also be due to an accumulation of NKT cells at tumour sites, thus decreasing their overall number in the circulating PB. This, together with longitudinal studies, aimed at ascertaining whether the low NKT cells numbers in these patients predated the manifestation of cancer, would help further the understanding of the relationship between NKT cell numbers and cancer.

There were insufficient subject numbers to ascertain treatment-related effects on NKT cells breast cancer patients, and indications that chemotherapy modulates NKT cell numbers in lung cancer patients cannot be conclusively determined with the subject numbers available in this subgroup.

The effect of the ageing process on NKT cell numbers has only been assessed in a singly study (DelaRosa et al, 2002). In accordance with that study, we showed a decreased percentage

![Figure 2](image-url)  
**Figure 2**  Expansion capacity of NKT cells in normal donors (n = 37) and cancer patients (n = 28) in response to α-GalCer stimulation (line indicates median of group). Natural killer T cells can rapidly expand in both groups, however overall to a significantly lower degree in cancer patients (P = 0.02).
In summary, our study demonstrates that NKT cell numbers and their activation and capacity can be modulated by both age and malignancy and in contrast to previous studies, conventional cancer treatment. Whilst further studies are required to determine both the stage at which treatment or malignant disease leads to decreased NKT cell numbers and the mechanism by which cancer affects impacts NKT cell function, our results provide important information for the clinical trials involving in vivo activation or large-scale in vitro expansion of NKT cells.

ACKNOWLEDGEMENTS

We thank Nirmala Pondeja for assistance with statistical analyses.

REFERENCES

Chouaib S, Asselin-Paturel C, Mami-Chouaib F, Cairns A, Blay JY (1997) The host–tumor immune conflict: from immunosuppression to resistance and destruction. Immunol Today 18: 493 – 497

Delarosa O, Taranzona R, Casado JG, Alonso C, Ostor B, Pena J, Solana R (2002) Valpha24+ NKT cells are decreased in elderly humans. Exp Gerontol 37: 213 – 217

Dellabona P, Padovan E, Casorati G, Brockhaus M, Lanzavecchia A (1994) An invariant V alpha 24-J alpha Q/V111 T cell receptor is expressed in all individuals by clonally expanded CD4-8- T cells. J Exp Med 180: 1171 – 1176

Goto S, Sato M, Koezuka Y, Nicol A, Nieda M, Juji T (1999a) Antitumor cytotoxicity mediated by ligand-activated human V24NKT cells. Cancer Res 59: 5102 – 5105

Kawano T, Tanaka Y, Shimizu E, Kaneko Y, Kamata N, Sato H, Osada H, Sekiya S, Nakayama T, Taniguchi M (1999) A novel recognition motif of human NKT antigen receptor for a glycolipid ligand. Int Immunol 11: 881 – 887

Keisling R, Wasserman K, Horiguchi S, Kono K, Sjoberg J, Pisa P, Petersson M (1999) Tumor-induced immune dysfunction. Cancer Immunol Immunother 48: 353 – 362

Kikuchi A, Nieda M, Schmidt G, Koezuka Y, Ishihara S, Ishikawa Y, Takodoko K, Durrant S, Juji T, Nicol A (2001) In vitro anti-tumour activity of z-galactosylceramide-stimulated human NKT cells in an in vivo model. Br J Cancer 85: 741 – 746

Mizoguchi J, O’Shea J, Longo DL, Loeffler CM, Picciotto MW, Stohla AC (1992) Alterations in signal transduction moledules in T lymphocytes from tumor-bearing mice. Science 258: 1795 – 1798

Mizoguchi J, O’Shea J, Longo DL, Loeffler CM, Picciotto MW, Stohla AC (1992) Alterations in signal transduction moledules in T lymphocytes from tumor-bearing mice. Science 258: 1795 – 1798

Natalie A, Koezuka Y, Porcelli SA, Ishikawa Y, Koezuka Y, Nicol A, Nieda M, Juji T, Fujisawa T, Shinkai Y, Taniguchi M, Juji T (1999) Activation of Valpha24+ NKT cells in human subjects results in highly coordinated secondary activation of acquired and innate immunity. Blood 25: 25

Ninomiya T, Akbar SM, Masumoto T, Horiike N, Onji M (1999) Dendritic cells with immature phenotype and defective function in the peripheral blood from patients with hepatocellular carcinoma. J Hepatol 31: 323 – 331

Plackett TP, Boehmer ED, Faunce DE, Kovacs EJ (2004) Aging and innate immune cells. J Leukoc Biol 23: 23

Sekiya S, Nakayama T, Taniguchi M, 1999a) Antitumor cytotoxicity mediated by ligand-activated human V24 NKT cells. Cancer Res 59: 5102 – 5105

Kawano T, Tanaka Y, Shimizu E, Kaneko Y, Kamata N, Sato H, Osada H, Sekiya S, Nakayama T, Taniguchi M (1999) A novel recognition motif of human NKT antigen receptor for a glycolipid ligand. Int Immunol 11: 881 – 887

Kawano T, Tanaka Y, Shimizu E, Kaneko Y, Kamata N, Sato H, Osada H, Sekiya S, Nakayama T, Taniguchi M (1999b) A novel recognition motif of human NKT antigen receptor for a glycolipid ligand. Int Immunol 11: 881 – 887

Kawano T, Tanaka Y, Shimizu E, Kaneko Y, Kamata N, Sato H, Osada H, Sekiya S, Nakayama T, Taniguchi M (1999b) A novel recognition motif of human NKT antigen receptor for a glycolipid ligand. Int Immunol 11: 881 – 887

Kawano T, Tanaka Y, Shimizu E, Kaneko Y, Kamata N, Sato H, Osada H, Sekiya S, Nakayama T, Taniguchi M (1999) A novel recognition motif of human NKT antigen receptor for a glycolipid ligand. Int Immunol 11: 881 – 887